DIABETIC PERIPHERAL NEUROPATHY AND RETINAL TISSUE THICKNESS

Sangeetha Srinivasan

M.Phil (Optometry)

Supervisors

Professor Nathan Efron

Dr. Nicola Pritchard

Submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

School of Optometry and Vision Science

Institute of Health and Biomedical Innovation

Faculty of Health

Queensland University of Technology

2014

Keywords

Diabetic peripheral neuropathy

Diabetic retinopathy

Ganglion cell complex

Neuropathy disability score

Retinal nerve fibre layer

Retinal thickness

Abstract

Diabetic peripheral neuropathy is a major cause of foot ulceration and lower limb amputation (Boulton, 2005). Recent studies demonstrated compromised neuroretinal anatomy (Shahidi et al., 2012), retinal function (Lövestam-Adrian et al., 2012) and mid-peripheral visual field sensitivity (Sampson et al., 2012) in relation to diabetic peripheral neuropathy. In view of the fact that macular and the foveal integrity are crucial for central vision, the structural integrity of the retina in the macular area deserves particular attention. To date, there is no information regarding the integrity of the macula in relation to diabetic peripheral neuropathy. Therefore, this research program examined retinal tissue thickness in relation to diabetic peripheral neuropathy. Full retinal thickness, inner retinal thicknesses at the macula (Ganglion Cell Complex, GCC) and that around the optic nerve head (Retinal Nerve Fibre Layer, RNFL) were examined.

Collective evidence from the past suggests key variables such as diabetic retinopathy (DR), age, sex of the individuals, duration of diabetes and HbA_{1c} levels may influence retinal tissue thickness. Therefore, this research program took into account the above key variables for all experiments.

The diabetic cohort comprised of individuals with type 1 or type 2 diabetes. However, it was unclear if the two cohorts can be combined to represent one group of individuals with diabetes. Therefore, as a preliminary step, the relationship between type of diabetes and retinal tissue thickness was explored. It was observed that individuals with type 2 diabetes had a tendency for reduced retinal thicknesses when compared to those with type 1 diabetes; however, this association was not statistically significant. Therefore, the groups with type 1 and type 2 diabetes were combined into one cohort. Subsequently, the primary research questions were explored.

Neuropathy status was defined using the Neuropathy Disability Score (NDS). The NDS involved neurological evaluation of three sensory modalities in the feet, namely, vibration sensation, hot and cold sensation, sharp and blunt sensation and also the Achilles tendon reflex; the outcome was recorded on a continuous scale from 0-10. An NDS ≥ 3 was defined as neuropathy, with higher NDS scores indicating advanced degrees of neuropathy. Thus, the association between retinal tissue thickness and the severity of neuropathy was assessed.

It was observed that the perifovea (outer macula) is thinner in relation to the severity of neuropathy. On the other hand, the parafovea (inner macula) is thinner in the presence of retinopathy.

As far as the inner retinal thickness is concerned, the RNFL is thinner in individuals with advanced degrees of neuropathy. The GCC thickness also showed a tendency to be reduced in relation to the severity of neuropathy; however, the relationship did not reach statistical significance. Interestingly, neither RNFL nor GCC thickness showed a significant relationship to DR, thus demonstrating that neuroretinal degeneration in diabetes is related to peripheral neuropathy rather than retinopathy.

A thinner perifovea may be associated with changes in the outer retinal layers and more specifically the photoreceptor layer. A thinner RNFL may be associated with loss in visual field sensitivity. Therefore, this necessitates investigation of visual function.

Examination of pattern-based GCC parameters revealed that individuals with diabetes have pockets of loss in GCC volume that is related to the severity of neuropathy and a generalized loss in GCC volume that is related to age. These findings were not related to DR, duration of diabetes, or the HbA_{1c} levels.

In conclusion, this research program has contributed to new knowledge regarding the retinal anatomy in relation to diabetic peripheral neuropathy. The full retinal tissue thickness, the macular and peripapillary nerve fibre layer thickness is decreased in individuals with diabetic peripheral neuropathy. The results of this research provide a better understanding of these changes in

relation to neural pathology occurring elsewhere in the body in individuals with diabetes. These findings have important implications to visual function, mobility and quality of life issues. More specifically, the retinal compromise documented here may pose a threat to the visual integrity of individuals with neuropathy, and hence investigation of the functional correlates of these structural changes (i.e. assessment of visual function) is warranted.

Thesis outline

The thesis is organized as follows:

Chapter 1 reflects on the natural history of diabetic neuropathy, the risk factors involved and the traditional tests used in the assessment of neuropathy. The final section of this chapter presents the ophthalmic complications in relation to diabetic neuropathy. The purpose of this chapter is to provide an in-depth understanding of the signs and symptoms in diabetic neuropathy and the tests involved in the detection of neuropathy.

Chapter 2 discusses in detail, retinal degeneration in diabetes. The intention of this chapter is to offer a fundamental insight into the vascular and neural involvement in diabetes. The chapter begins with a brief introduction to retinal anatomy and the factors influencing retinal anatomy. Following this a detailed discussion of the features of diabetic retinopathy is presented. The literature that investigated retinal thickness in individuals with diabetes has been reviewed and debated in the later sections of Chapter 2.

Chapter 3 presents the general methodology used in this research. Methodology specific to each individual experiment has been provided in respective chapters.

The experiments constitute Chapters 4 to 6. A series of cross-sectional studies examined the relationship between diabetic peripheral neuropathy and the retinal tissue thickness in a group of individuals with type 1 or type 2 diabetes. The full retinal and the inner retinal thicknesses were examined in relation to diabetic neuropathy. Further study involved examination of pattern-based ganglion cell complex parameters in relation to diabetic neuropathy and is discussed in Chapter 5. Chapter 6 presents an analysis of the retinal tissue thickness in individuals who had undergone laser treatment for diabetic retinopathy, in comparison to those with retinopathy but no laser treatment.

Finally, Chapter 7 summarizes the findings from all the experiments, including a discussion of the strengths, limitations and the implications of this work.

Table of contents

List of Tables

List of Figures

Conference presentations

1. Srinivasan S, Pritchard N, Edwards K, Russell AW, Efron N. Retinal tissue thickness is reduced in diabetic peripheral neuropathy. World Diabetes Congress, 2013, Melbourne, Victoria, Australia.

2. Srinivasan S, Pritchard N, Sampson G, Edwards K, Shahidi AM, Efron N. Inner retinal tissue thickness in diabetes. Institute of Health and Biomedical Innovation (IHBI) Inspires Postgraduate Conference, 2011, Brisbane, Queensland, Australia.

List of Abbreviations

BMI	Body mass index
BP	Blood pressure
CV	Conduction velocity
DM	Diabetes mellitus
DME	Diabetic macular oedema
DNSS	Diabetic neuropathy symptom score
DPN	Diabetic peripheral neuropathy
DR	Diabetic retinopathy
EDB	Extensor digitorum brevis
ERG	Electroretinogram
ETDRS	Early Treatment Diabetic Retinopathy Study
FH	Fossa head
FLV	Focal loss volume
GCC	Ganglion cell complex
GLV	Global loss volume
H/Ma	Haemorrhages/Microaneurysms
HbA_{1c}	Glycosylated haemoglobin
IOP	Intra ocular pressure
IRMA	Intraretinal microvascular abnormalities
LGN	Lateral geniculate nucleus
NDS	Neuropathy disability score
NGSP	National glycohaemoglobin standardization program
NPDR	Nonproliferative diabetic retinopathy
NVD	Neovascularisation at disc
NVE	Neovascularisation elsewhere
OCT	Optical coherence tomography
ONH	Optic nerve head
PDR	Proliferative diabetic retinopathy
QST	Quantitative sensory testing
RMS	Root mean square
RNFL	Retinal nerve fibre layer
SD-OCT	Spectral domain optical coherence tomography
T1DM	Type 1 diabetes
T2DM	Type 2 diabetes

Statement of Original Authorship

The work contained in this thesis has not been previously submitted to meet requirements for an award at this or any other higher education institution. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person except where due reference is made.

QUT Verified Signature

Signature:..

Date: 19/06/2014

Achievement

The candidate won a Third Prize at the QUT Faculty of Health, 3-Minute Thesis Competition held on 28th August 2012.

Acknowledgements

I would like to thank everyone who has helped me directly or indirectly during this rewarding journey.

My sincere thanks to QUT for providing me with the financial support, without which, this dream would not have been achievable. I am indebted to the participants of this study without whom this work would not have been possible.

I express my sincere thanks to Associate Professor Peter Hendicott, Head of School, School of Optometry and Vision Science. I express my sincere gratitude to my Principal Supervisor, Professor Nathan Efron. His constant encouragement and support has always been a motivation for me. It has been a privilege to work under his supervision. I owe my special thanks to Dr. Nicola Pritchard, my Associate Supervisor, for her guidance and support right from my day one in Brisbane and throughout my candidature. Thanks to Dr. Katie Edwards for her support and cheerful attitude.

Words cannot express my gratitude to Dr. Dimitrios Vagenas, who has always been there whenever I approached him for statistical advice. My sincere thanks to Dr. Geoff Sampson; I learnt many valuable research skills through his interaction.

I would like to thank my colleagues at the LANDMark study who have helped me during this journey.

I thank my parents and my friends for their support.

Words cannot express how much I have missed my beautiful little son, who has been waiting for me back home, while I came overseas to pursue my PhD. I thank God for giving me the courage and strength to pursue this endeavour.

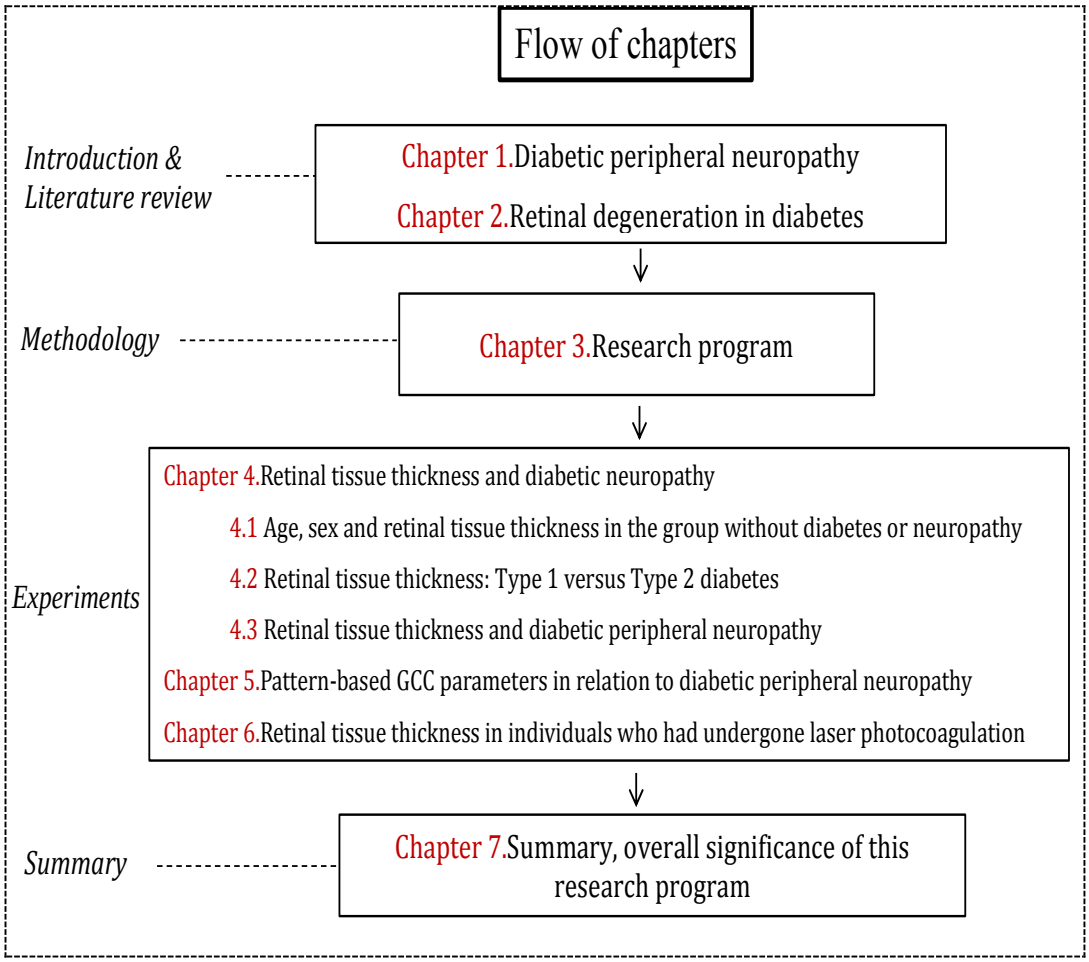


Figure 1. A schematic representation of the flow of chapters

Chapter 1: Diabetic peripheral neuropathy

Diabetes is the leading cause of non-traumatic lower limb amputation (Siitonen et al., 1993). Diabetic peripheral neuropathy (DPN) predominantly affecting the sensory nerves of the feet and lower limbs can be particularly problematic as it can lead to foot ulceration and eventually amputation (Boulton, 2005). Corneal sensory nerve networks have been shown to be compromised in relation to the degree of peripheral neuropathy (Malik et al., 2003) (Quattrini et al., 2004) (Tavakoli et al., 2010). This link between diabetic peripheral neuropathy and the eye has been also demonstrated by alterations to retinal anatomy (Shahidi et al., 2012) and visual fields (Sampson et al., 2012). These two studies demonstrated peri-papillary nerve fibre layer thickness reduction and mid-peripheral visual field sensitivity reduction in people with neuropathy compared to people without neuropathy. In view of the fact that macular and the foveal integrity are crucial for central vision, the structural integrity of the retina in the macular area deserves particular attention. Therefore, this research project was designed to explore retinal tissue thickness in people with and without diabetic neuropathy in relation to other factors that affect the retinal thickness. Further rationale is discussed in Chapter 3. As it is important to gain an in-depth understanding of diabetic neuropathy and the risk factors involved, this chapter begins with an introduction to diabetic neuropathy, followed by a review of pathogenesis in neuropathy. Subsequently, the natural history of diabetic neuropathy, risk factors, and the diagnostic tests for diabetic neuropathy are described. Ocular changes in association with diabetic neuropathy are subsequently discussed.

1.1 Introduction

Peripheral neuropathy can affect up to 50% of individuals with diabetes (Tesfaye et al., 1996). The aetiology has been linked to prolonged raised blood glucose levels and compromised blood supply in diabetes. Initially, the

peripheral nerves are involved with the first sign of nerve damage occurring in the feet (Reiber et al., 1998). If left untreated, it can affect other body parts.

A person with neuropathy can have symptoms such as numbness or tingling at one end of the spectrum or painful symptoms at the other end of the spectrum (Boulton et al., 1998). Loss of sensation in the foot can increase the risk of foot injury. This when combined with poor wound healing in diabetes, can lead to foot ulcers and may require amputation.

Neuropathic pain can be mild or severe but can worsen especially at night (Archer et al., 1983). This causes the person to seek medical attention. It can also cause unsteadiness while walking and can interfere with daily activities. Symptoms can also include poor bladder control, bowel movement or impaired movement of one or more toes. Since no effective treatment exists, screening for the disease and early intervention becomes vital, in arresting the development or in preventing or delaying the progression of neuropathy.

1.2 Pathogenesis

Several mechanisms such as hyperglycaemia, polyol pathway, ischaemia and oxidative stress have been identified in the pathogenesis of diabetic neuropathy (Tomlinson, 2002). A schematic has been shown in Figure 2. A combination of the above pathways has been proposed in the pathogenesis of diabetic peripheral neuropathy.

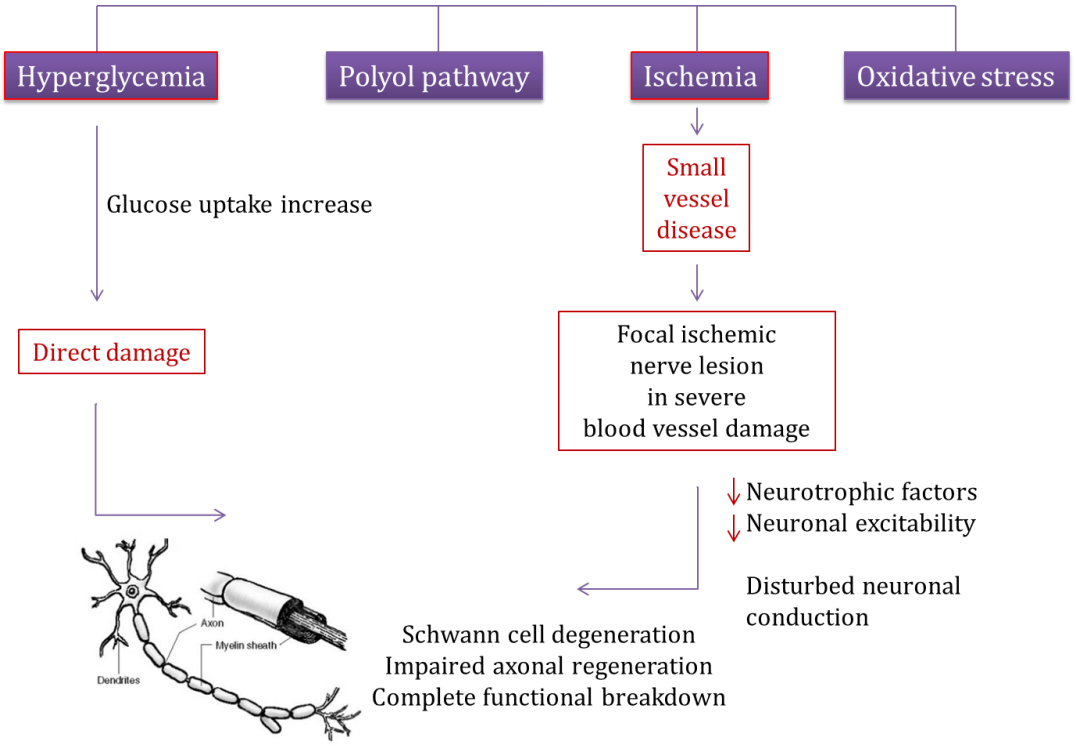


Figure 2. Hyperglycaemia, polyol pathway, ischaemia and oxidative stress are some of the pathways identified in the pathogenesis of diabetic neuropathy

A chain of events is involved, that lead to eventual axonal degeneration and a functional breakdown.

There is a more direct damage to the neurons from hyperglycaemia and ischaemia. Neurons need a constant supply of glucose; glucose uptake depends on extracellular glucose concentration. In the presence of hyperglycaemia, there can be up to a four-fold increase in the uptake of glucose. When this condition occurs long-term or occurs more frequently, the nerves are damaged.

The enzyme aldose reductase, catalyses the conversion of glucose to sorbitol. Peripheral nerve damage has been reported to be associated with over-expression of aldose reductase inhibitor enzyme in animal models (Yagihashi et al., 2001). High extracellular glucose increases oxidative metabolism of glucose in the mitochondria, which releases free radicals (Glock et al., 1955). There is Schwann cell damage and a reduction in a range of factors such as free radical scavengers and nerve growth factors, along with a decrease in the regenerative

capacity of axons. These changes can ultimately damage cellular properties resulting in direct damage to the nerve axons.

Nerves depend on small blood vessels for their nutrition and oxygen supply. High blood glucose levels affect the blood vessels that supply the nerves and consequently the nerves are damaged. Focal nerve lesions have been observed with severe blood vessel damage. However, neuronal function improved with improvement in blood supply thus indicating a strong association (Cameron et al., 2001) and also a potential to improve with intervention. There is also an overall reduction in neurotrophic factors like nerve growth factor and a subsequent decrease in the ability to repair nerves and degeneration of Schwann cells, followed by a complete neuronal functional breakdown.

Certain other factors such as C-peptides, VEGF, immune mechanisms and insulin-like growth factors have also been reported to be involved in the pathogenesis of diabetic neuropathy (Boulton et al., 2004). It is encouraging that neuropathy is responsive to modifications in the glycaemic levels and improvement in blood supply thus demonstrating a potential for improvement.

1.3 Natural history of diabetic neuropathy

The natural history of diabetic peripheral neuropathy is not well defined because the criteria for diagnosis and the methods for detection varied between studies and therefore the prevalence. Diabetic neuropathy can be thought to present in two ways: a gradually progressive type that worsens with the duration of diabetes and the other acute type that can be treated. Neuropathy may present as 1) Peripheral neuropathy 2) autonomic neuropathy (Vinik, 2008) 3) focal neuropathy 4) proximal neuropathy or a combination of these. The following sections examine in depth, the type of nerve fibre involvement, the symptoms in neuropathy and the traditional tests used in the detection of neuropathy. Peripheral neuropathy and autonomic neuropathy are the commonly occurring types and are therefore discussed in detail in the following sections.

1.3.1 Types of nerve fibres

Presentation of neuropathy can largely depend on the type of nerve fibres involved. The nerve fibres can be broadly classified as large, medium and small fibres (Brown et al., 2004). The features are summarized below.

Large nerve fibres

- Heavily myelinated
- A-alpha fibres (motor strength)
- A-beta fibres (vibratory, touch sensation)

Medium nerve fibres

- Myelinated
- A-gamma fibres (information to muscle spindles)

Small nerve fibres

- Myelinated A-delta fibres
- Unmyelinated or myelinated C fibres
- Both innervate skin (somatic fibres), involuntary muscles (cardiac, smooth muscles, autonomic fibres)

A-alpha fibres are large and heavily myelinated and are responsible for motor strength and proprioception. A-beta fibres are also heavily myelinated but carry vibratory and touch sensations. A-delta fibres are small myelinated fibres that carry sensory information about pain and cold sensation. A-gamma fibres carry information to muscle spindles.

C-fibres are small fibres that may be myelinated or unmyelinated and carry sensation of warmth and pain and also regulate autonomic reflexes such a blood pressure, heart rate regulation and sweating.

Figure 3 is a representation of the type of neuropathy and the corresponding regions affected.

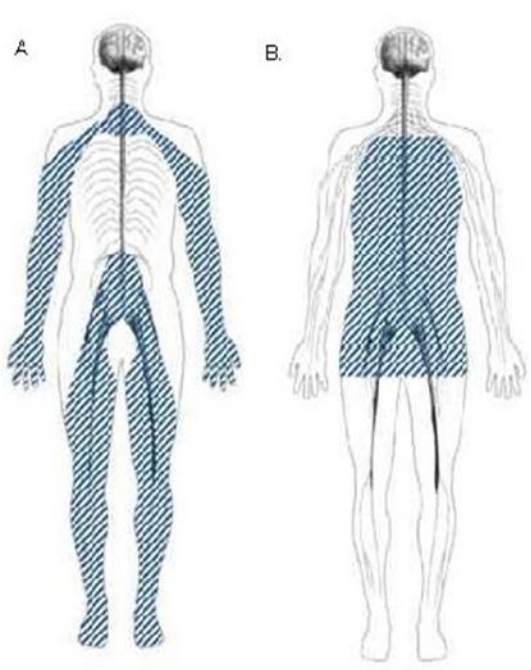


Figure 3. (A) Peripheral neuropathy (B) Autonomic neuropathy. Adapted from Diabetic neuropathies: The nerve damage of diabetes, National Diabetes Information Clearinghouse

1.3.2 Peripheral neuropathy

This is also referred to as distal polyneuropathy; the most common neuropathic presentation in both type 1 and type 2 diabetes, characterised by a length-dependent process that involves distal nerves first and then progresses to involve proximal nerves; also called as glove-stocking distribution. Figure 3A is a representation of regions involved. By the time, knees are affected, symptoms progress to the tips of fingers (Veves et al., 2007, p.251). Impaired sensation can progress to involve the skin of the chest.

Neuropathic symptoms

Symptoms are primarily sensory with tingling or numbness and tend to be symmetrical (Boulton et al., 2009) thus indicating large nerve fibre involvement.

Symptoms such as burning sensation or pain in the lower extremities (Lacomis, 2002) suggests small fibre involvement. Patients can have abnormal or impaired thermal sensation but essentially normal vibration perception.

Paradoxically, individuals with painful neuropathy may have only a minimal neurological deficit but individuals with loss of sensation in the foot can have very advanced stage of nerve damage (Boulton, 2005) thus emphasizing the need for a more comprehensive neuropathy evaluation. However, the presence of pain in neuropathy is thought to be protective (Boulton, 2012) where individuals can have heightened awareness of the problem and can approach the health practitioner and have their diabetes status monitored.

1.3.3 Autonomic neuropathy

Cardiac neuropathy and postural hypotension are some of the features of autonomic neuropathy and are explained below. Resting tachycardia (where an individual's heart rate during rest is abnormally high or more than 100 beats per minute) is generally the first clinically detectable sign of cardiac neuropathy (Stewart et al., 1992). A fall in systolic blood pressure of 20 mmHg or more, when switching from lying to standing position, without any increase in heart rate is defined as postural hypotension (Stewart et al., 1992). There can be a risk of silent ischemia and myocardial infarction in people suffering from cardiac autonomic neuropathy. Gastroparesis (signs of fullness or vomiting), diabetic diarrhoea (watery diarrhoea after food especially at night), bladder atony (large residual volume after microurition), impotence and premature ejaculation are some disconcerting symptoms in autonomic neuropathy (Said, 2007). Figure 3B is shows regions involved in autonomic neuropathy. Ophthalmic manifestations of autonomic neuropathy may involve a sudden painful ophthalmoplegia (extra ocular muscle paresis), abnormal pupillary light reflexes or miosis (Lanting et al., 1990).

Non-diabetic causes of neuropathy

Other causes of peripheral neuropathy include autoimmune diseases like rheumatoid arthritis (Pallis et al., 1965), infections such as Hansen's disease (Charosky et al., 1983), AIDS (Simpson et al., 2000), certain toxic substances like alcohol (Hillbom et al., 1984), vitamin deficiency (McCombe et al., 1984), and chronic exposure to radiation, trauma, and tumours exerting pressure on the

spinal cord. A detailed history can therefore play a vital role in differential diagnosis.

1.4 Risk factors for diabetic neuropathy

Prolonged duration of diabetes, poor glycaemic control, advancing age, systemic hypertension, smoking, higher body mass index, abnormal lipid profile and alcohol intake have been reported as risk factors for DPN (Adler et al., 1997; Forrest et al., 1997; Partanen et al., 1995; Tesfaye et al., 2005; Van De Poll-Franse et al., 2002). Of these, poor glycaemic control and prolonged duration of diabetes have been reported as strong risk factors.

In another study (described below), the proportion of people with neuropathy was higher among those with prolonged duration of diabetes. The prevalence of neuropathy was 12% among those with duration of diabetes less than 7 years and the percentage increased to 42% for duration of 15 years and greater. A similar trend was also observed for higher HbA_{1c} values. The proportion of people with diabetic neuropathy was 26% for a HbA_{1c} level of 5.4-6.4%, and the percentage increased to 40% for a HbA_{1c} value greater than 7.8% (Tesfaye, et al., 1996). In addition, treatment of chronic hyperglycaemia led to improvement or reversal of neurological abnormalities and symptomatic relief from pain, thus suggesting a strong association between hyperglycaemia and diabetic neuropathy (Boulton, 1982).

Tesfaye et al (1996) examined the frequency of diabetic neuropathy in a cohort of people with type 1 diabetes. The authors observed the prevalence of diabetic neuropathy to be lower (19%) in the younger age group 15-29 years, as compared to that in the age group 45-61 years (57%), when adjusted for duration of diabetes and HbA_{1c} suggesting that advancing age is an important risk factor in diabetic neuropathy.

The relative risk for developing neuropathy has been reported to be 3.92, 95% CI [2.23, 6.91] for the presence of systemic hypertension; 1.74, 95%CI [1.07, 2.83] for positive history of smoking and 2.6, 95% CI [1.6, 4.3] for HbA_{1c} levels of ≥ 10% (Forrest et al., 2002).

The question whether the type of diabetes is related to neuropathy is not well understood. Prevalence of neuropathy has been reported to be slightly higher in people with type 2 diabetes (32.1%) compared to that in type 1 diabetes (22.7%) in a multicentre study conducted in hospital population in the UK (Young et al., 1993). In experimental animal models, neuropathy has been reported to differ between the two types of diabetes. Kamiya et al (2004) and Sima et al (2006) observed greater extent of axonal degeneration in type 1 form of rats than the type 2 form of rats, (Kamiya et al., 2005) (Sima et al., 2006). Therefore, the relationship between type of diabetes and neuropathy is not well understood and requires further investigation.

For the purposes of this research project, risk factors namely age, duration of diabetes, HbA_{1c} levels, systemic hypertension, smoking, body mass index and lipid profile have been analysed in individuals with and without neuropathy.

1.5 Diagnostic tests for diabetic neuropathy

Traditional tests for diabetic neuropathy include nerve conduction studies, quantitative sensory testing (QST) and autonomic nerve function testing (Tesfaye et al., 2009). The nerve conduction studies, 128-Hz tuning fork, ankle reflex tests and monofilament generally test for the large nerve fibres involvement; temperature sensation assessment using hot and cold rods and pinprick sensation evaluation using Neurotip® device (as a part of NDS) are generally used in the assessment of small nerve fibre involvement. Neuropathy symptoms are evaluated using the diabetic neuropathy symptom score (DNSS).

The QST, NDS, monofilament test and the DNSS are subjective in nature in that, the participants are required to respond; whereas, skin biopsy, nerve conduction studies, Neuropad and heart rate variability are objective tests for neuropathy. Evaluation of postural changes in blood pressure, the heart rate variability and sweating response is a part of autonomic neuropathy testing. The following sections describe in detail, the tests involved in the assessment of neuropathy.

1.5.1 Skin biopsy

Skin biopsy involves excising a 3mm section on the skin of the distal leg and thigh (Polydefkis et al., 2001). This allows for a direct and a quantitative evaluation of the peripheral nerve fibres but requires qualified medical personnel. In addition, only small fibre involvement can be assessed. Nevertheless, skin biopsy can also reveal information about morphological changes such as swelling and pathological changes in the intraepidermal nerve fibres (IENF) such as branched patterns, which are difficult to visualize otherwise. The IENF also inversely correlates with duration of diabetes and elevated warm sensation threshold (Shun et al., 2004). Despite the advantage over other techniques, skin biopsy can still be debated as a minimally invasive technique. However, several other non-invasive techniques (described below) have emerged that involve non-invasive assessment of both small and large fibre involvement and can be performed in a clinical scenario with minimal training or specialization.

1.5.2 Diabetic neuropathy symptom score (DNSS)

Diabetic neuropathy symptom score is a validated questionnaire for symptom assessment that contains four general questions regarding unsteadiness while walking, experience of burning and aching pain or tenderness sensation in legs or feet, prickling sensation in legs or feet and places of numbness in legs or feet. A score of 1 is given for the presence of symptom and 0 for the absence of symptom. A score of 1 or more out of 4 is abnormal (Meijer et al., 2002).

1.5.3 The 10g Monofilament test

This is a sensitive, reproducible and a rapid screening method to assess touch/pressure sensation in large nerve fibres (Smieja et al., 1999) (Dros et al., 2009) (Perkins et al., 2010). The 10g monofilament test enables the clinician to map the areas of reduced pressure perception by exerting a specific repeatable bending force on test site (Figure 4) and by having the participant respond whether they felt it or not (Valk et al., 1997). The outcome is recorded as number of points detected out of three.

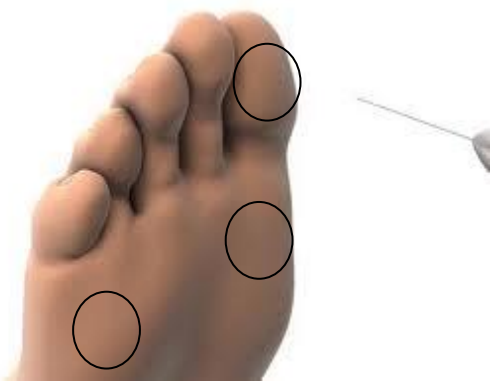


Figure 4. Monofilament test: Circles showing tested sites

(David et al., 2005)

1.5.4 Neuropathy disability score (NDS)

The NDS test involves neurological examination of three sensory modalities namely, sharp and blunt sensation, hot and cold sensation and vibration sensation. In addition, the ankle reflex is also tested. Figure 5 shows the instruments used for NDS assessment.

Sharp and blunt sensation is tested using a Neurotip® device for both touch and pain sensation. A neurotip device has two ends: one end with a sharper tip to test for pain sensation and a blunt tip to test for blunt sensation. The other end has the monofilament (described earlier). The individual is asked to identify if the type of stimulus is sharp or blunt (Figure 5A).

The temperature sensation is assessed in the arch of the feet using rods immersed in hot and cold water (Figure 5B).

Vibration perception is tested with a 128-Hz tuning fork on the plantar aspect of the great toe and the patient responds with a 'yes' or 'no' if they felt it or not (Figure 5C).

A score of 0 is given for correct response and 1 for incorrect identification for each individual test component in the above three tests.

The ankle reflex is assessed using a reflex hammer, with the scores being 0 for normal, 1 for reinforcement and 2 for absent (Figure 5D).

Each foot can have maximum score of 5 resulting in a total score of 10 for both feet. NDS scores 0-2 is classified as no neuropathy, 3-5 indicates mild neuropathy, 6-8, moderate neuropathy and 9-10 indicates severe neuropathy. NDS score of six or greater predicts foot ulceration (Young et al., 1993). The NDS criterion of neuropathy has been utilized in the current study.

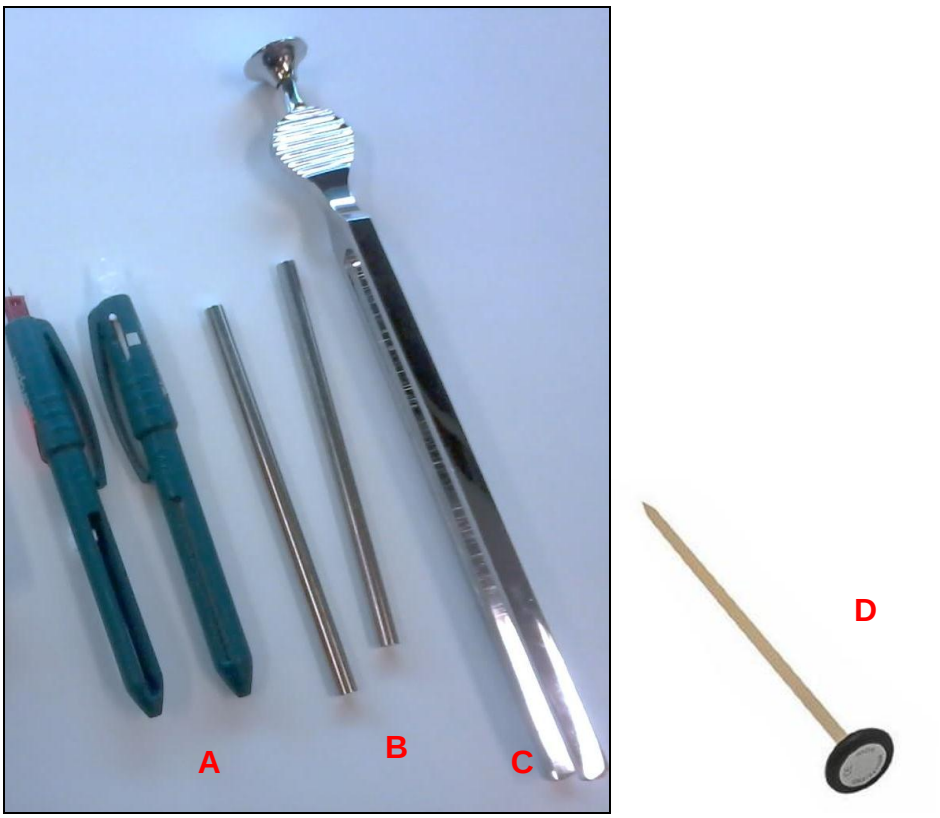


Figure 5. Equipment used in NDS testing (A) Neurotip® device (B) Hot and cold rods (C) Tuning fork (D) Reflex hammer

1.5.5 Nerve conduction studies

The nerve conduction studies involve electrophysiological assessment of peroneal, tibial or sural nerve conduction velocities (CV) and amplitudes in the lower limb of the participants (Figure 6), in order to assess the degree of damage to the nerve fibres in relation to neuropathy. Nerve conduction

velocities and amplitudes are then compared with the age-related normal values. Nerve conduction studies (NCS) have been reported as a sensitive method for detecting peripheral neuropathy (Valensi et al., 1997). However, NCS essentially tests the large nerve fibre involvement and therefore are not very helpful in detecting small fibre neuropathy (Jamal et al., 1987); in addition, the testing requires trained examiners.

Figure 6. Electrophysiology testing

1.5.6 Quantitative sensory testing

Quantitative sensory testing (QST) is a set of valid quantitative psychophysical methods for evaluating sensory nerve function. QST measures the threshold in response to a range of stimuli such as vibration and thermal stimuli and pain thresholds in response to heat and cold (Young et al., 1994).

Figure 7 shows the Neurosensory Analyser Model TSA-II utilized in the current study.

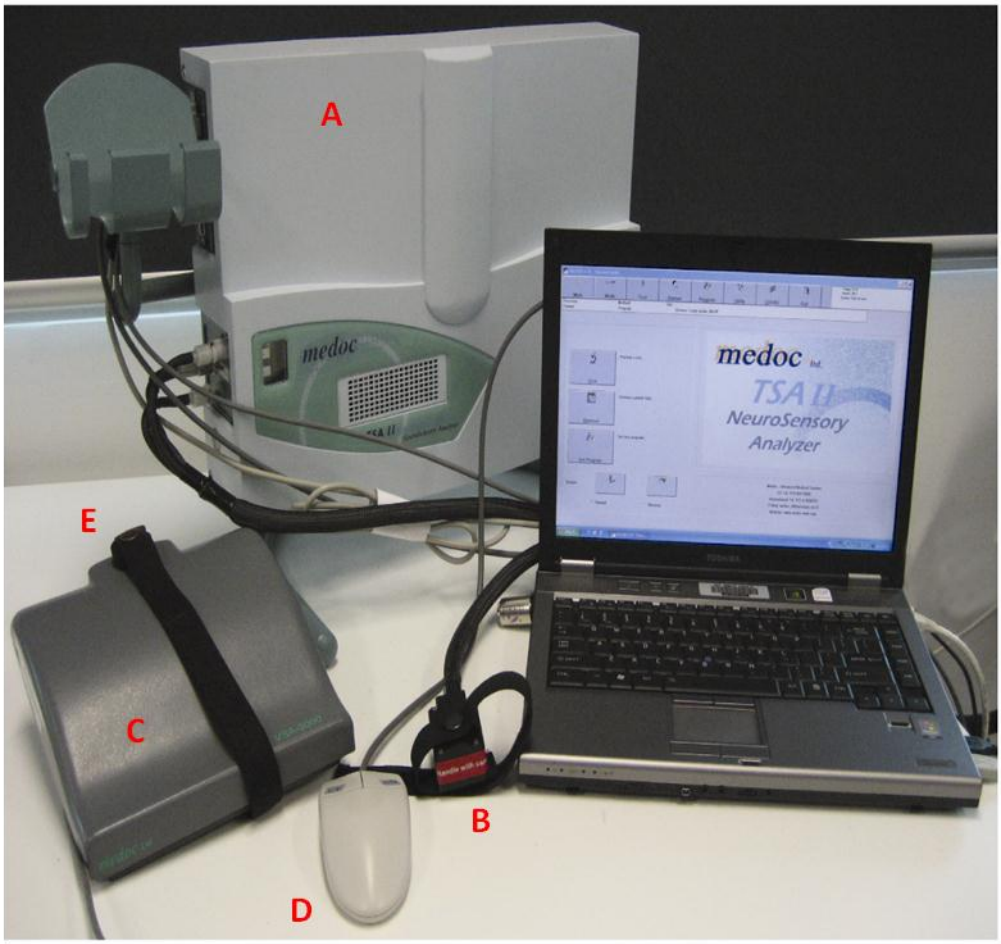


Figure 7. The Neurosensory Analyser Model TSA-II (A) Neurosensory analyser (B) Thermode (C) Vibratory sensory analyser (D) Patient response button (E) Vibration stimulator (Image: courtesy Dr. Ayda M Shahidi)

The threshold values are then compared with age and gender-based normative values. With the variation in the type of stimulus used, QST can detect large and small fibre deficits. (Vinik et al., 1995). In the presence of sensory deficit, the threshold will be elevated. However, QST requires the use of sophisticated equipment and may be time consuming in a clinical environment. In addition, the subjective nature of the test requires the person to be cooperative and attentive.

Since QST involves quantitative evaluation of small and large nerve fibres, QST can be of great value in assessing neuropathic progression (Zaslansky et al., 1998). The current research program has analysed neuropathy variables as assessed using QST measures.

It is to be noted that there is an ongoing debate regarding the best method to detect neuropathy. For small fibre neuropathy, however, skin biopsy is accepted as the standard technique. There is currently no accepted gold standard test for detecting diabetic peripheral neuropathy (Horowitz, 2002).

1.5.7 Tests for autonomic neuropathy

Assessment for postural hypotension (Ewing et al., 1982) (as previously described in section 1.3.3), heart rate variability and Neuropad™ are some of the tests used in autonomic nervous system testing.

Heart rate variability (HRV) involves assessing beat-to-beat alterations in heart rate during deep breathing with the use of electrocardiograph equipment (Osterhues et al., 1998).

The Neuropad™ (miro Verbandstoffe GmbH, Wiehl, Germany) is a simple, rapid and an inexpensive test for autonomic neuropathy that assesses small fibre dysfunction, by assessing the sympathetic cholinergic innervations to the skin. Sympathetic nerve fibres supply sweat glands. Neuropad™, an adhesive pad containing cobalt salts, is attached to plantar aspect of the foot. The pad changes colour from blue to pink within 10 mins if sudomotor and hence the cholinergic sympathetic function is normal (Stewart, et al., 1992). An abnormal neuropad response is associated with sympathetic dysfunction and clinical neuropathy (Quattrini et al., 2008).

For the purposes of this thesis, measures of HRV and Neuropad are not included.

It is to be noted that there is an ongoing debate regarding the best method to detect neuropathy. To date, there is no accepted gold standard for detecting diabetic peripheral neuropathy (Horowitz, 2002).

Diabetic neuropathy has been reported to be associated with neuronal complications in the eye. The following section discusses the studies reporting this association.

1.6 Corneal and retinal changes in relation to diabetic neuropathy

1.6.1 Corneal nerve structure and corneal sensitivity compromise

Various corneal nerve structural and functional deficits have been reported in relation to diabetic neuropathy. Corneal sensitivity and vibratory perception in the distal leg were found to be reduced in people with diabetes and has been attributed to diabetic polyneuropathy (Nielsen, 1978). Corneal nerve fibre density and nerve fibre branching (Edwards et al., 2012; Edwards et al., 2012; Efron et al., 2010) are significantly reduced in people with diabetes when compared to people without diabetes (Malik, et al., 2003; Quattrini, et al., 2004). Corneal nerves are tortuous in people with severe neuropathy when compared to mild and moderate degrees of neuropathy as well as the group without diabetes (Kallinikos et al., 2004). A corneal sensitivity threshold of 0.66 millibars or higher with non-contact corneal aesthesiometry most likely indicates neuropathy (Pritchard et al., 2012; Pritchard et al., 2010). Corneal nerve fibre growth has been demonstrated after pancreatic transplantation (Mehra et al., 2007). Corneal nerve fibre density improved after a 25-month metabolic control (Iqbal et al., 2005), suggesting improvement in nerve structure with intervention. This emphasizes the importance of early detection for timely referral so as to prevent further damage. These studies suggest an association between a generalised form of diabetic neuropathy and nerve fibre changes in the cornea (Edwards et al., 2012, Edwards et al., 2012; Efron et al., 2010; Pritchard et al., 2011). Apart from the corneal compromise, retinal investigations also revealed compromised retinal structure, function and visual field sensitivity in association with diabetic neuropathy. The following studies report this finding.

1.6.2 Retinal structure and visual functional compromise

Sampson et al (2012) analysed the visual field sensitivities in people with type 2 diabetes with neuropathy (defined using NDS), in comparison to people with diabetes without neuropathy. Visual field sensitivities were analysed by quadrant and at eccentricities 0-10, 11-20 and 21-30 degrees using standard

automated perimetry. The authors noted similar visual sensitivities in the two groups for smaller degrees of eccentricity. However, for eccentricities beyond 7 degrees, there was a steep relative decline in visual field sensitivity in the neuropathy group. Also at eccentricities beyond 15 degrees, the groups with and without DPN were statistically shown to be separated. The difference between groups was greatest for eccentricity 21-30 degrees (Sampson et al., 2012).

A previous pilot study by Shahidi et al (2012) assessed the relationship between retinal nerve fibre layer (RNFL) thickness and peripheral neuropathy in a group comprising of 82 individuals with type 2 diabetes and compared with 24 healthy individuals. Participants were in the age group 45-77 years, with no difference in age or male to female ratio in either group. The diabetes cohort consisted of 23 people with no neuropathy (NDS 0-2), 32 with mild neuropathy (NDS 3-5), 16 with moderate neuropathy (NDS 6-8) and 11 with severe neuropathy (NDS 9-10). An NDS score ≥ 6 was used for defining people at risk of foot ulceration (Abbott et al., 1998). There were 36 individuals without DR, 16 with minimal DR, 25 mild DR, and 1 moderate DR (Shahidi et al., 2012). Global and sectoral RNFL thicknesses were examined with a spectral domain OCT in people stratified according to neuropathy disability score. The authors noticed no difference in RNFL global or sectoral thicknesses between people with diabetes with and without neuropathy. However, for people at risk of foot ulceration (NDS ≥ 6), the inferior RNFL thickness was significantly reduced.

Multivariable regression models were used to determine the relationship between neuropathy and RNFL thickness. There was no effect of retinopathy or interaction between retinopathy and neuropathy on RNFL thickness. The study observed that the RNFL thickness reduced with the severity of neuropathy defined using NDS criteria. The reduction in RNFL thickness was not explained by age, glycaemic levels or the retinopathy status, in a cohort of people with type 2 diabetes.

Another recent study observed significantly reduced multi-focal visually evoked potential (mfVEP) amplitudes in the lower nasal visual field quadrant, in people

with neuropathy, compared to those without neuropathy (Lövestam-Adrian et al., 2012). The mfVEP is an objective method for assessing neuroretinal function (Jiang et al., 2011). It is interesting to note that both the groups had similar percentage of people with DR; however the group with neuropathy were significantly older (mean difference = 12 years) than the group without neuropathy. For the reason that advancing age is a risk factor for neuropathy in people with diabetes, the differences in age may have confounded the results to a certain extent. Nevertheless, this could also suggest a local response of a generalised polyneuropathy that might be exerting subtle neuroretinal changes in the eye that is not explained by DR.

In addition to the inner retinal layer compromise, it is likely that the other retinal layers may be involved in diabetic neuropathy. Therefore, with the knowledge obtained from Shahidi's study, the current study was designed to explore the full retinal thickness, macular and the peripapillary nerve fibre layer thickness in individuals with and without neuropathy. Collective evidence from previous studies indicate that several factors such as DR, age, sex, duration of diabetes and HbA_{1c} levels influence the retinal tissue thickness. In the current research project, multivariable regression models have been utilized to adjust for the known confounding factors, for example, age and DR.

Having discussed the features associated with diabetic neuropathy, Chapter 2 will focus on retinal degeneration in diabetes.

Chapter 2: Retinal degeneration in diabetes

2.1 Introduction

Several studies (described below) have attributed neuroretinal degeneration in diabetes to diabetic retinopathy. However, other studies have observed degeneration in retinal layers even in the absence of DR. More recently, compromised RNFL thickness (Shahidi et al., 2012), decreased visual field sensitivity (Sampson et al., 2012), and visual function in the inferior quadrant (Lövestam-Adrian et al., 2012) in individuals with diabetes have been attributed to diabetic neuropathy rather than DR. This leads to the further hypothesis that the retinal tissue thickness in diabetes reported in previous studies may have been confounded by diabetic peripheral neuropathy. With this knowledge, the current research program has been structured around the hypothesis that DPN may be related to retinal tissue thicknesses and therefore may confound estimates of retinal tissue thickness in studies that fail to account for the possible impact of DPN.

This chapter presents a review of the retinal anatomy, and discusses the factors that influence retinal tissue thickness. Subsequently, studies that have examined retinal anatomy and function in the presence and the absence of diabetic retinopathy are reviewed.

2.2 Retinal anatomy

The macula is located between the superior and the inferior arcades temporal to the optic nerve head, and slightly below the horizontal meridian (Antonetti et al., 2006). Spitznas M (1973) (as cited by Apple, 1981) reported that the entire macula measures about 5.5 mm in diameter; the centre of the macula the fovea, measures approximately 1.5 mm in diameter and has a bright foveal reflex at the centre due to the concavity of the foveal pit that allows it to act as a concave mirror (Rose, 1988). The central 500 μm of the fovea is called the foveal avascular zone (FAZ) and is totally devoid of blood vessels and therefore

dependent on the choriocapillaris for its nutrient supply (Tombran-Tink et al., 2007.pg.18). The foveola (centre of the fovea, measuring about 0.2 mm diameter) has sloping walls called clivus which is seen as an annular ring reflex in children and young adults (Hildebrand et al., 2010). The foveola has the highest density of cone photoreceptors, where the rods are essentially absent. The long axons of the photoreceptors that form the Henle's layer fan out from the centre (also called the parafovea) (Chan et al., 2006).

Polyak (1941) was the first to describe the macula as fovea, parafovea and perifovea (Polyak, 1941). A schematic of this has been shown in Figure 8A. The fovea lies within the imaginary circle of diameter 1mm; the parafovea is the region outside the 1.5 mm and within 2.5 mm diameter. The parafovea comprises of several layers of ganglion cells. On the other hand, the perifovea marks the periphery of the macula where the density of ganglion cells is comparatively lesser and is the region outside the 2.5 mm zone but within 5.5 mm circle. The peripheral retina purportedly lies outside the temporal arcades where the ganglion cell layer is essentially single layer in thickness.

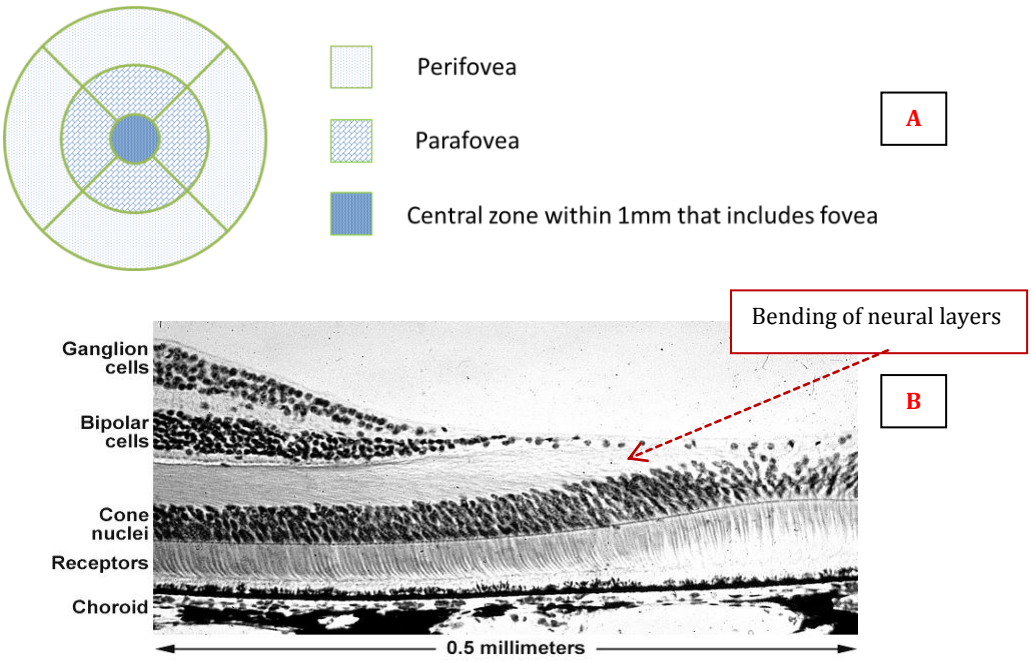


Figure 8. (A) Concentric retinal zones namely central zone, parafovea, perifovea (B) Histology of retina near fovea. (Image adapted from the University of Delaware Image Library)

The retina comprises of 10 layers. The retinal pigment epithelium (RPE) which is the outermost retinal layer (Figures 8B and 9), adjacent to the choroid, is a single layer of cuboidal epithelium containing melanin, and is densely packed with mitochondria, endoplasmic reticulum and ribosomes (Fantes et al., 1989). Lipofuscin, which is a product of degradation of outer segments of photoreceptors, is also present in the RPE cells. Photoreceptors (rods and cones) are specialized neuronal cells that convert light impulses to nerve signal by a process called transduction.

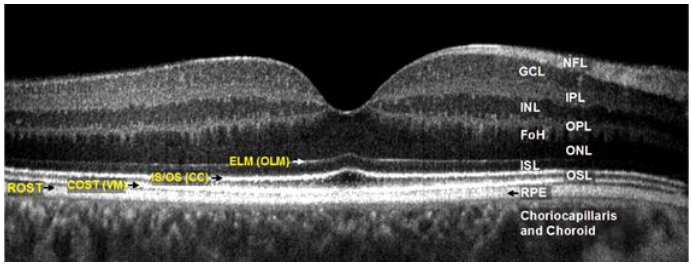


Figure 9. Retinal layers as seen on SD-OCT (Werner et al., 2011)

The human retina contains 3-6 million cones and 75-107 million rods. Electron-microscopic structure of a photoreceptor comprises of an outer segment that contains photopigment, an inner segment, a filament that resembles an axon and an end terminal that connects to the outer plexiform layer. The terminologies, 'rods' and 'cones' are derived from the shape of the outer segments of photoreceptors. The photoreceptor nuclei constitute the outer nuclear layer, which is the thickest in the foveolar region (Figure 9).

The retina is composed of three layers of nerve cell bodies and two layers of synapses (Figure 9).

The layers comprising of the nerve cell bodies are:

(i) Outer nuclear layer (ONL) (consisting of nerve cell bodies of rods and cones)

(ii) Inner nuclear layer (INL) (consisting of nerve cell bodies of amacrine cells, horizontal cells and bipolar cells)

(iii) Ganglion cell layer (GCL) (consisting of nerve cell bodies of ganglion cells) (Lens et al., 2008)

The two layers of synapses are, the

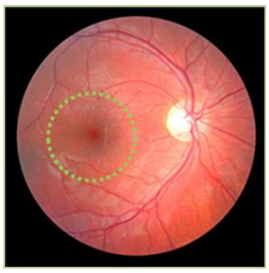
(i) Outer plexiform layer (OPL) (has synapses between endplates of rods and cones with that of the dendrites of amacrine, horizontal and bipolar cells)

(ii) Inner plexiform layer (IPL) (has synapses between the axons of bipolar cells and the dendrites of ganglion cells (Figure 9). This also has interspersed network of vertically and horizontally directed amacrine cells that integrate with ganglion cells (Krebs et al., 1991).

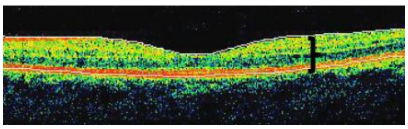
The photoreceptor terminals interact with each other via the horizontal and bipolar cells (also called interneurons). These interneurons which also include amacrine cells, connect the photoreceptor layer to the ganglion cells and hence the nerve fibre layer. Bipolar cells connect as little as one cone with that of ganglion cell, whereas, they connect many rods with each ganglion cell more peripherally. The fibres (axons) of the ganglion cells constitute the retinal nerve fibre layer. There are four types of glial cells in the retina; Muller cells, astrocytes, microglia and oligodendrocytes (Bringmann et al., 2013). Muller cells extend to the outer and deeper layers of the retina, to form the outer and inner limiting membranes respectively. Endothelial cells, pericytes, astrocytes and amacrine cells also occupy the inner retinal layers. Research reports twenty or more different ganglion cell types (Shabana et al., 2003). The axons of the ganglion cells travel towards the optic nerve head. However, due to their convergence at the optic nerve head (ONH), the nerve fibre layer is the thickest around the ONH (Curcio et al., 1990).

2.3 Factors influencing the full retinal thickness

The full retinal thickness is essentially the thickness from the retinal pigment epithelium up to the vitreoretinal layer (Sadda et al., 2006) (Figure 10). The full retinal tissue thickness is influenced by factors such as age, gender, refractive error and ethnicity. The following section provides a review of those findings.



full retinal thickness



Full retinal thickness – influenced by

1. Age
2. Gender
3. Ethnicity
4. Refractive error and other clinical parameters

Figure 10. Factors affecting the full retinal thickness (indicated by black line)

The studies cited in this entire review used optical coherence tomography (OCT) technique unless otherwise stated.

2.3.1 Retinal thickness and age

Total macular volume (volume data of the macular area) as well as the macular thickness has been found to decrease with age (Fraser-Bell et al., 2005).

The Early Treatment Diabetic Retinopathy Study (ETDRS) observed that the thickness in the nine zones thicknesses reduced at the rate of 0.26-0.46 μm per year increase in age (Eriksson et al., 2009). Figure 8A shows a schematic of ETDRS zones.

Retinal thickness reduces at the rate of 0.53 μm per year increase in age (Alamouti et al., 2003). In contrast, a group of authors observed no relationship with age (Wong et al., 2004). Differences in the scan protocols could explain the variations in study results. Alamouti et al used a vertical scan line of 2.3 mm aligned at temporal disc margin. However, Wong et al observed thickness measured at the central 1 mm macula to have no relationship with age. In general, the reduced macular thickness with age might possibly be related to

age-related ganglion cell loss and hence the axons (RNFL). For the reason that the neural layers are essentially pushed away from the foveal centre, any changes in the neural layers with age may not be noticeable in the foveal centre. Ooto et al (2011) noticed the thickness between inner-outer segment of photoreceptor till the inner border of photoreceptors were positively correlated with age meaning that the foveal thickness increased with age; the authors hypothesized that increase in foveal thickness with age may be due to reduced RPE phagocytosis (Ooto et al., 2011). Therefore, the current study has taken into account, the effect of age.

2.3.2 Retinal thickness in males and females

It is unclear if the sex of the individual is related to retinal thickness. Studies in the past have observed thickness in the macular area to be greater in healthy men than women (Massin et al., 2002) (Wang et al., 2004) (Bressler et al., 2008) (Huang et al., 2009) whereas other studies found that macular thickness was similar in male and female participants (Lattanzio et al., 2002) (Chan et al., 2006). Foveal thickness has been reported to be greater in males than females (Adhi et al., 2012) but in another study, the foveal thickness has been reported to be similar between men and women (Chan et al., 2006). Although there is some disagreement in the literature regarding the effect of sex of an individual and the retinal thickness, it is likely that sex of the individuals does play a role in retinal thickness; therefore the current project has taken into account the sex of the individuals, with respect to full retinal and inner retinal thicknesses.

2.3.3 Retinal thickness and ethnicity

Asians and African-Americans (Kashani et al., 2010) have thinner macula compared to Caucasians (Kelty et al., 2008). African-Americans have a tendency for reduced foveal thickness and significantly reduced macular thickness compared to Caucasians (Asefzadeh et al., 2007). Interestingly, RNFL thickness (described in section 2.4) has been reported to be thicker in Hispanics and in Asians compared to Caucasians (Bundez et al., 2007). The current study

examined the ethnic composition in the cohort of people with and without diabetes for the retinal thickness assessments.

2.3.4 Retinal thickness, refractive error and other clinical parameters

Fraser-Bell et al (2005) reported reduced total macular volume for myopia greater than -5.00 D. Intraocular pressure and keratometry readings were not associated with retinal thickness (Fraser-Bell, et al., 2005). However, longer axial lengths and larger body mass index were associated with thicker retina within the central 1 mm (Wong et al., 2004). Foveal thickness was positively associated with the axial length (Sato et al., 2010) but the macular thickness measurements in all quadrants were inversely correlated to the axial length (Lam et al., 2007).

In the current research program, the relative effects of the known confounding variables have been adjusted by utilising multiple regression models.

2.4 Factors influencing the inner retinal thickness

The inner retinal layers that include the RNFL and GCC thickness (Figure 11) are influenced by certain variables and are described in the following section.

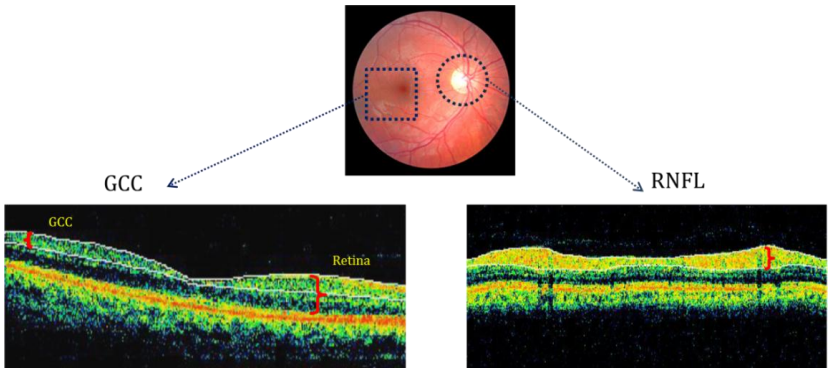


Figure 11. Factors affecting inner retinal thickness

Inner retinal thickness – influenced by

1. Age
2. Axial length
3. Ethnicity
4. Gender

RNFL and GCC thicknesses reduce with advancing age. The RNFL reduces at the rate of 2 μm, 95% CI [1.2, 2.8 μm] per decade in Caucasians (Budenz et al., 2007) and Indian eyes (Malik et al., 2012). It has been reported that GCC thickness reduces at the rate of 1.59 μm per decade increase in age in a group of Korean adults (Kim et al., 2011) (Koh et al., 2012).

The RNFL thickness is related to axial length. RNFL thickness has been found to decrease by 2.2 μm (Bundez et al., 2007) and 1.27 μm (Koh et al., 2012) for every 1 mm increase in axial length. RNFL thickness has been reported to be related to the optic disc size. For every 1 square mm increase in optic disc area, the RNFL thickness has been observed to increase by 3.3 μm (Budenz, et al., 2007).

The RNFL thickness also varies across different ethnic groups. Bundez et al (2007) observed thicker RNFL in Asian adult eyes compared to those of Hispanics or Caucasian adults.

Difference in RNFL thicknesses between males and females has not been observed so far. However, a recent study (described below) observed females to have thinner GCC when compared to males. Koh et al (2012) observed the ganglion cell complex-inner plexiform layer thickness to be thinner in females (2.3 μm thinner GCC than males).

Variations in retinal thickness have been reported in people with diabetes, in the presence and absence of clinical signs of DR.

2.5 Diabetic retinopathy and its influence on retinal tissue thickness

2.5.1 Introduction

Diabetic retinopathy (DR) has been reported to confound full retinal and inner retinal thickness investigations. The purpose of this review is to enumerate the findings from previous studies that examined retinal tissue thicknesses using imaging techniques, both in the presence and the absence of DR.

Diabetic retinopathy (DR) remains the leading cause of visual impairment in working-age adults (Porta et al., 2002). The National Health and Medical Research Council (Australia) defines diabetic retinopathy by the presence of typical retinal microvascular lesions in a person with diabetes. Once the stage of severe retinopathy is established, any further improvement in glycaemic levels, as in pancreatic or renal transplantation, does not improve retinopathy (Ulbig et al., 1991). Therefore, it is vital to detect early signs and identify modifiable risk factors so as to prevent the worsening or to begin intervention in the early stages of diabetic retinopathy.

The following section provides an introduction to the clinical features of diabetic retinopathy, the stages and the risk factors involved in the development and progression of retinopathy.

2.5.2 Prevalence

According to the American Diabetes Association, one-third of individuals with diabetes will develop DR at some stage. The National Health and Medical Research Council in its guidelines for management of DR (Mitchell et al., 2008), states that people with type 2 diabetes may have DR at the time of diagnosis, whereas in type 1 diabetes, DR is not so common within five years of diagnosis. An age-standardized prevalence has been reported to be 34.6% for preproliferative DR, 6.9% for proliferative DR, 6.1% for diabetic macular oedema, and 10.2% for vision-threatening DR in individuals with type 2 diabetes (Yau et al., 2012). This was determined from a meta-analysis of prevalence rates of DR from 35 studies conducted worldwide.

The prevalence rates of DR were calculated among those with type 1 diabetes; Roy and his group using the data from two population-based studies conducted in the United States, the New Jersey 725 study and the Wisconsin Epidemiologic study of diabetic retinopathy. The two studies examined people with type 1 diabetes older than 18 years. Nearly 75% of African-Americans had some form of DR and 30% had vision-threatening retinopathy. Among Caucasians, 82% had some form of DR and 32% had sight-threatening DR (Roy et al., 2004).

It has been estimated that the number of people with any stage of DR would increase from 126.6 million in the year 2011 to 191 million by the year 2030, and the prevalence of vision-threatening DR would increase from 37.3 million to 56.3 million if not attended to immediately (Zheng et al., 2010). Therefore, a clear understanding of the clinical features and risk factors involved in DR is essential for a timely intervention; this may help to slow down or decelerate the changes that lead to the development or the progression of diabetic retinopathy thus preserving the existing retinal anatomy and function and thereby maintain visual integrity.

2.5.3 Clinical features and stages

Based on the Airlie House classification (Davis et al., 1969), diabetic retinopathy can be broadly staged as follows:

a. Background diabetic retinopathy (BGDR)
b. Proliferative diabetic retinopathy (PDR)

Background diabetic retinopathy (BGDR) - also called nonproliferative DR (NPDR)

This represents an early stage of DR characterised by the appearance of microaneurysms, dot and blot haemorrhages, capillary occlusion and hard exudates (each of these signs is explained in detail below). NPDR can be further classified into mild, moderate, severe and very severe NPDR based on certain characteristics. A brief description has been provided in Table 1.

Proliferative diabetic retinopathy (PDR)

This is an advanced stage of DR characterised by the appearance of abnormal new blood vessels, fibrovascular tissue, vitreous haemorrhage, and tractional retinal detachment (Davis, 1992).

The Early Treatment Diabetic Retinopathy Study (ETDRS) modification (Davis et al., 1998) of the Airlie House classification is employed as a staging system for

the DR in a majority of clinical trials. This has been used in the current research program to define the stages of retinopathy.

Table 1 shows the ETDRS classification and staging system for diabetic retinopathy.

Table 1. ETDRS staging and classification system for diabetic retinopathy. Table derived from Davis et al., 1998

Level	Severity	Definition
10	No retinopathy	Diabetic retinopathy absent
20	Very mild NPDR	Micro-aneurysms only
35	Mild NPDR	Hard exudates, cotton-wool spots, and/or mild retinal haemorrhages
43	Moderate NPDR	43A Retinal haemorrhages moderate (>photograph If) in four quadrants or severe (≥photograph 2A) in one quadrant 43B Mild IRMA (<photograph 8A) in one to three quadrants
47 A -D	Moderate NPDR	47A Both level 43 characteristics 47B Mild IRMA in four quadrants 47C Severe retinal haemorrhages in two to three quadrants 47D Venous beading in one quadrant
53 A-D	Severe NPDR	53A ≥2 level 47 characteristics 53B Severe retinal haemorrhages in four quadrants 53C Moderate to severe IRMA (≥photograph 8A) in at least one quadrant 53D Venous beading in at least two quadrants
53E	Very Severe NPDR	≥2 level 53A-D characteristics
61	Mild PDR	NVE<0.5 disc area in one or more quadrants
65	Moderate PDR	65A NVE≥0.5 disc area in one or more quadrants 65B NVD <photograph 10A (<0.25-0.33 disc area)
71, 75	High-Risk PDR	NVD ≥photograph 10A, or NVD <photograph 10A or NVE<0.5 disc area plus VH or PRH, or VH or PRH obscuring ≥1 disc area
81, 85	Advanced PDR	Fundus partially obscured by VH and either new vessels upgradable or retina detached at the centre of the macula

NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; IRMA, intraretinal microvascular abnormalities; NVE, new vessels elsewhere; NVD, new vessels on or within 1-disc diameter of the optic disc; PRH, pre-retinal haemorrhage; VH, vitreous haemorrhage. (This classification does not cover macular oedema or photocoagulation)

The following are some of the features in diabetic retinopathy. The photographs were obtained from http://www.ucdenver.edu/academics/colleges/medicalschool/centers/BarbaraDavis/Clinical/Pages/Ophthalmology.aspx

Microaneurysms (MA) occur in the early stages of DR and appear as small red dots (Kohner, 1978). MA's represent out pouching of the capillary blood vessel wall (Mahon et al., 1971) located in inner nuclear layer of the retina (Ashton, 1969). These lesions are sometimes not distinguishable from the dot and blot haemorrhages. However, the margins of MA's are clearly demarcated than those of the haemorrhages. Figure 12 shows ETDRS Standard photograph 1 with microaneurysms.

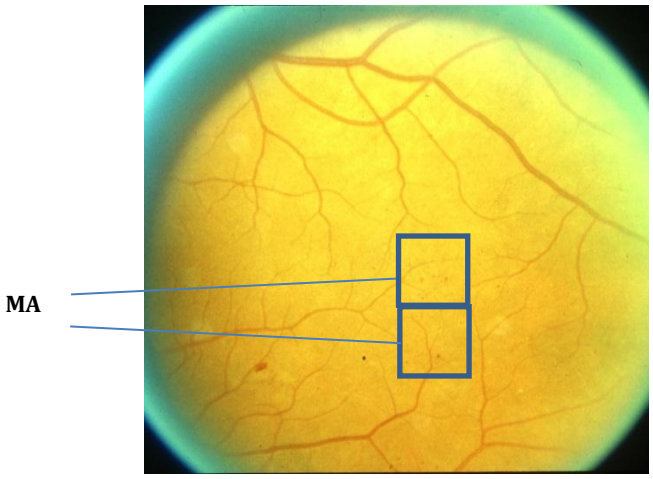


Figure 12. ETDRS Standard photograph 1.Microaneurysms

Retinal haemorrhages (H's) represent sites of bleeding from blood vessel, either from a ruptured aneurysm or from increased permeability of the blood vessel wall. These haemorrhages can be a dot, blot (arising from venous end of capillaries that are essentially in the deeper retinal layers) or flame-shaped (arising from precapillary arteriole which are essentially in the retinal nerve fibre layer) (Ashton, 1949). Haemorrhages do not have distinct margins as do the MA's. However, dot haemorrhages tend to have borders that are more distinct than blot haemorrhages. H's may take 3 or 4 months to resolve.

Distinguishing between MA and H's clinically is not crucial, as they both are present in early stages of DR. Figure 13 shows ETDRS standard photograph 2A that has both, MA's and H's.

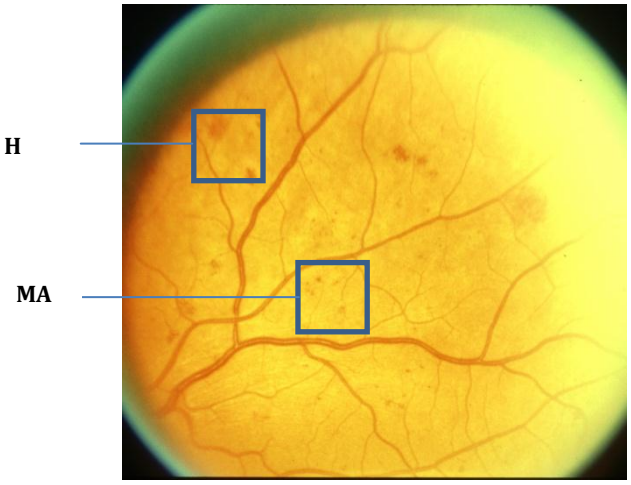


Figure 13. ETDRS Standard photograph 2A. Retinal haemorrhages and microaneurysms

Hard exudates (HE) represent deposition of lipids in the outer plexiform layer of the retina, due to leakage of plasma containing lipoprotein, crystalloid and water (Ferris et al., 1996) resulting from vasodilation. Exudates tend to be smaller ranging from 15-55 µm. They often accompany macular oedema where they arrange themselves in circinate or circular pattern (Bernard et al., 1995), at the junction of oedematous and normal retina (Singh et al., 2008). Figure 14 is the ETDRS Standard photograph 4 showing hard exudates.

Figure 14. ETDRS Standard photograph 4. Hard exudates

ETDRS classified macular oedema as (1) clinically non-significant oedema and (2) clinically-significant macular oedema (CSME) (ETDRS, 1985); CSME is defined as one or more of the following:

(i) retinal thickening at or within 500 μm of the centre of the macula; hard exudates at or within 500 μm of the centre of the macula, if associated with adjacent retinal thickening;
(ii) a zone or zones of retinal thickening one disc area in size, at least part of which is within one disc diameter of the centre of the macula;
(iii) thickening greater than 1 disk diameter in size, part of which is within 1 disk diameter of centre or macula.

Soft exudates or "cotton wool spots" (CWS) are white, cottony lesions that represent infarcts, or ischemic changes within the nerve fibre layer. Cotton wool spots often occur in association with intraretinal microvascular anomalies (IRMA) (discussed below).

Intraretinal microvascular anomalies (IRMA) are spider-like branched vessels that appear generally in the later stages of NPDR. They are often tortuous and convoluted in appearance. IRMA is considered as either dilated capillaries that already exist or formation of new blood vessels within the retina.

Venous Beading appears during the same stages like the IRMA, where the vessels lose their normal shape and they represent contracted columns

resembling sausage-like segments. Figure 15 is the Standard photograph 6B showing IRMA and venous beading (ETDRS, 1985).

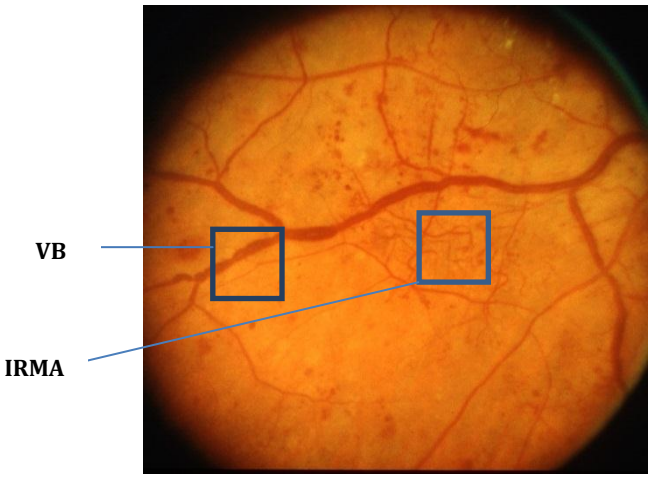


Figure 15. ETDRS Standard photograph 6B. Venous beading, Intra retinal microvascular abnormalities

New vessels defined as neovascularisation at the disc (NVD) and neovascularisation elsewhere (NVE) are the first of the proliferative lesions to appear. The theory is that during conditions of hypoxia, the retina compensates by growing new vessels in the areas lacking circulation. The new vessels are delicate and tend to grow on top of the retina rather than into it, making them prone to leakage and haemorrhage. They can cross over one or many major retinal blood vessels, but tend not to cross over themselves. They often appear in a blossom like a flower bud with the outside part of the NV more dilated than the inner NV. They can also appear as stringy and drawn out, but this presentation is not common (Davis, 1969).

2.5.4 Risk factors for diabetic retinopathy

People with diabetes will develop DR at some stage. Later stages of DR such as severe NPDR and clinically significant macular oedema have visual implications. Therefore, it is essential to monitor for the known and the modifiable risk factors.

Duration of diabetes is one of the strong risk factors for the development and progression of DR, but it is not modifiable. Higher glycaemic level, poor blood

pressure control and dyslipidaemia are identified as strong risk factors in the development of DR (Ulbig et al., 1993) (Yau et al., 2012). Glycaemic level has been found to be positively related to the incidence and progression of retinopathy, after controlling for duration of diabetes, age, gender and baseline retinopathy findings (Klein et al., 1988). However, glycaemic level (HbA_{1c}) is a modifiable risk factor for DR. There exists a possibility that the progression of DR can be slowed or prevented by achieving optimal glycaemic levels. The Australian Government, in its NHMRC guidelines (2008), recommends that the HbA_{1c} levels be maintained below 7% for people with DR. In addition, blood pressure greater than 130/85 mmHg in people with diabetes is considered a risk factor for DR (Yam et al., 2007). Normalizing blood pressure and serum lipid levels has beneficial effects especially in cases of diabetic macular oedema in DR, (Ferris et al., 1996). Pregnancy, puberty and anaemia have been reported as other risk factors for DR (Agardh, 2002; Moloney et al., 1982; Sunness, 1988). There is also an ongoing debate about the role of nonhealing ulcers (Harris Nwanyanwu et al., 2013), obesity, smoking, alcohol consumption and physical inactivity (Mohamed et al., 2007) in the progression of DR.

2.5.5 Monitoring and treatment

Traditional methods of treatment include strict control of HbA_{1c} levels, blood pressure and lipid levels and regular retinal evaluation and monitoring of retinopathy status (Neely et al., 1998).

Other forms of treatment include laser treatment, surgery for vitreous haemorrhage combined with complex retinal surgery as required. Advice on monitoring procedures depends on the severity of the condition. In minimal NPDR, procedures such as colour photography, fundus angiography or laser treatment is not advised. In mild NPDR, colour photography may be helpful for comparison with baseline findings, as there is a risk of progression to proliferative stage. For mild to moderate NPDR with CSME, OCT may be helpful in identifying subtle oedema or monitoring exiting CSME (American Academy of Ophthalmology Preferred Practice Guidelines).

2.5.6 Retinal degeneration in diabetic retinopathy

Diabetes is associated with alterations in multiple layers of the retina (Imai et al., 2009). For instance, there is evidence of loss of pericytes (de Oliveira, 1966) and vascular endothelial breakdown (Craitoiu et al., 2005) leading to vascular leakage (Aguilar et al., 2003; Antonetti et al., 1999; Feit-Leichman et al., 2005; Matsuura et al., 1976) and consequently diabetic retinopathy (Antonetti, et al., 2006); the above changes may be related to glial cell dysfunction and glial cell loss consequently leading to neuronal cell death (Gaucher et al., 2007).

Significance of glial cells

The astrocytes, Müller cells and microglia constitute the glial cell population. Under normal conditions, the astrocytes stem from the optic nerve (Watanabe et al., 1988) and travel towards the internal limiting membrane; astrocytes closely associate with neural elements and blood vessels (Gariano et al., 1996), protect the neurons from toxic (Chen et al., 2001) or anoxic (Vibulsreth et al., 1987) environments and also play a role in the maintenance of the blood-retinal barrier (Abbott et al., 2006; Gardner et al., 1997). Muller cells extend radially to form the internal and the external limiting membranes; Müller cells associate with the neurons to provide nutrition (Newman, 1987) and also regulate synaptic release or the uptake of GABA and glutamate neurotransmitters (Mizutani et al., 1998). The microglia is derived from monocytes that play a vital role in immunity. These cells secrete inflammatory cytokines and some neuroprotective substances that provide immunological (Milder, 2006) and mechanical support to the neuronal and vascular cells (Zeng et al., 2000); together, the glial cells aid in nutritional, immunological, supportive and protective functions for the neurons and the associated blood vessels. Therefore, the glial cells constitute an integral part of normal functioning of neurons and blood vessels. The glial cells are destroyed in diabetes. This affects the normal functioning of the neurons (Gardner et al., 2002; Hogan et al., 1963). In addition, the thickness in the outer and inner nuclear layers are reduced in animal models of diabetes, as evidenced by significant thinning at 10 weeks after inducing diabetes in mice (Martin et al., 2004); atrophy of synaptic

terminals is also reported in diabetic rodents (Scott et al., 1986). In human diabetes, reduced nerve fibre diameter and degeneration of nerve fibre layer, possibly from atrophy of ganglion cells, the axons, and dendrites (Barber et al., 1998; Lieth et al., 2000), have been observed.

These changes may manifest as changes in the retinal thickness profile. The retinal anatomical changes may be associated with a compromise in visual function as the neural cells and glial cells are important for maintaining vision (Lasansky, 1965).

2.5.6.1 Retinal structural and visual functional changes in diabetic retinopathy

Optic nerve head in the presence of retinopathy

Previous study observed that the size and shape of the optic disc, peripapillary atrophy and neuroretinal rim determined by morphometric analysis of optic disc photographs did not differ significantly in varying degrees of retinopathy when compared to that of those without diabetes (Königsreuther et al., 1995). However, a trend of decreased visibility of nerve fibre layer and increased disc pallor in eyes with increasing severity of retinopathy was noted; it was reported that optic disc in diabetic eyes can exhibit a non-glaucomatous appearance. The study had carefully excluded potential confounders such as high myopia -8.00 D or more, those diagnosed to have glaucoma as well as subjects with intra ocular pressure > 21 mmHg. Chihara et al (1998) compared optic nerve head (ONH) parameters in participants with and without diabetes. Despite the RNFL defects, individuals with diabetes had cup-disc ratios that were not significantly different when compared to that of individuals without diabetes (Chihara et al., 1998).

Visual function in the presence of retinopathy

In patients with retinopathy, a blue-yellow colour vision defect was observed but only in less than three percent of their subjects. The total number of deviations from the normal colour sequence on the FM-100 Hue test increased

with increasing severity of DR (Green et al., 1985). Another study examined visual field sensitivity in people with and without DR. Among those eyes classified to have very mild and mild DR, only 4% of eyes had mean deviation (MD) values with a probability value worse than 5% (Henricsson et al., 1994), suggesting that a vast majority of subjects with and without DR had MD within normal limits. However, the number of abnormal test points became higher with greater severity of DR.

Full retinal thickness in the presence of retinopathy

The following studies utilized imaging techniques to examine the retinal thickness in individuals with diabetic retinopathy. The full retinal thickness is essentially the thickness from the retinal pigment epithelium up to the vitreoretinal layer (Sadda, et al., 2006). Previous studies have observed thicker fovea, nasal and temporal parafoveal retina (Goebel et al., 2002), greater thickness in at least two ETDRS zones (Massin et al., 2002), thicker overall macula (Oshima et al., 1999) and thicker central zone (within 1mm diameter that includes the fovea) (Cho et al., 2010) in people with DR compared to those without diabetes; a trend of increase in macular thickness with increasing severity of DR has also been observed (Lattanzio, et al., 2002; Park et al., 2011). It has been postulated that in DR, there could be leakage of serum proteins into the intraretinal spaces following increased vascular permeability (Ehrlich et al., 2010). The increased vascular permeability may be one possible explanation for the increased macular thickness in DR (Davis, 1992).

In contrast, a large multi-centric study examined the central subfield thickness (the average thickness at all points within 1 mm diameter of fovea) in people with diabetes with no or minimal DR in comparison to data published in literature on people without diabetes and observed similar thicknesses in both groups (Bressler, et al., 2008). The difference in results reported in literature may also reflect variations in the presentation of DR as no two individuals with DR have same clinical features nor do they progress similarly (Vaz, 2011).

Inner retinal thickness in the presence of retinopathy

The inner retina predominantly comprises of neural elements such as neural cells, neurons and the glia (Barber et al., 1998). Histological sections of human cadaveric eyes with DR by Wolter (1961) and Bloodworth (1962) (as cited in Barber, 1998) showed neural degeneration and atrophy of ganglion cells and other neural elements with progressive loss of neural elements and a loss in visual function (Barber, et al., 1998).

However, with the advent of imaging techniques, it has become possible to examine in-vivo, the structural changes in eyes with retinopathy. Inner retinal thickness is significantly thinner in people with type 1 diabetes with no or minimal DR in the zone immediately around the fovea in comparison to group without diabetes (van Dijk et al., 2009; van Dijk et al., 2010). With scanning laser polarimetry, retinal nerve fibre layer thinning has been observed in eyes with preproliferative retinopathy (Oshitari et al., 2009); these in-vivo studies suggest neurodegenerative changes despite only mild degrees of DR. In addition, it was observed that the RNFL thinning worsens with more advanced degrees of retinopathy (Takahashi et al., 2006). With more recent versions of OCT where segmentation of various layers has become possible, in-vivo retinal examination reveals compromised ganglion cell and the inner plexiform layer thickness in people with minimal DR when compared to that of age and sex-matched people without diabetes (Biallosterski et al., 2007) (van Dijk et al., 2012). The compromised inner retinal thickness has been hypothesized as being related to hyperglycaemia-induced neuronal damage or ischaemic changes leading to consequent loss of ganglion cells in diabetic retinopathy (Park et al., 2010).

Summary of section 2.5.6.1

A frequently reported finding in literature is that in the presence of DR, the full retinal thickness increases with increasing severity of retinopathy, while, the inner retinal thickness decreases.

2.5.6.2 Retinal structural and visual functional changes in the absence of retinopathy

Several studies (discussed below) demonstrated neuroretinal compromise in the absence of clinically visible signs of diabetic retinopathy. The following section discusses studies that have documented structural and functional deficits in the absence of clinical signs of DR.

Optic nerve head in the absence of retinopathy

The ONH features have been studied in eyes without retinopathy. The ONH volumetric parameters that included rim and cup measures and RNFL thickness measures obtained using scanning laser ophthalmoscope in diabetic eyes without DR were not significantly different to that of people without diabetes (Tekeli et al., 2008; Toprak et al., 2012). In addition, the mean cup-disc ratio (vertical & horizontal) in diabetic eyes was not different from that of the controls (Park, et al., 2011).

Visual function in the absence of retinopathy

The following independent studies observed disturbances in certain visual functional parameters assessed by means of colour vision, contrast sensitivity and visual field testing.

Colour vision

Individuals with diabetes but without DR had tritan (blue-yellow) colour vision defects (Ong et al., 2003). When compared to those without diabetes, the error in colour discrimination was significantly higher in a group of people with diabetes without DR (Green, et al., 1985) (Hardy et al., 1992). However, it is likely that the yellowing of the crystalline lens may have confounded the results as there is the increased absorption of wavelength at the blue end of the spectrum.

Contrast sensitivity

In the absence of clinical signs of DR, contrast sensitivity (CS) has been reported to be compromised despite normal visual acuity in diabetic children (Georgakopoulos et al., 2011) and in diabetic adults (HyvÄRinen et al., 1983) (Heravian et al., 2010) (Trick et al., 1988); a trend of further reduction in CS was observed in individuals with early to more advanced DR (Ismail et al., 1998). The explanation behind impaired contrast sensitivity in diabetes is multi-fold; one of the several explanations being hyperglycaemia. Although no direct cause-effect relationship could be established between hyperglycaemia and CS, increased retinal blood flow has been associated with impaired CS during alterations in blood glucose levels (Bursell et al., 1996), thus suggesting an indirect link between the higher glycaemic levels and CS. Another likely explanation could be alterations in retinal and choroidal blood supply in diabetes that have been linked with impaired CS (Shoshani et al., 2011). Loss in CS has been associated with capillary dropout observed in individuals with diabetes (Fletcher et al., 2005). Other likely explanations for impairment in CS in individuals with diabetes may be related to diffraction of light due to pupillary miosis or intraocular scattering of light due to cataractous lenses.

Visual fields

Early study by Roth (1969) reported scotomas in the visual fields in people with diabetes, with and without DR. In the DR group, the scotomas topographically corresponded to retinopathy lesions. However, in the no DR group, the presence of scotomas was considered as a pre-retinopathy sign and was thought to be related to disturbances in retinal blood circulation (Roth, 1969).

Studies on type 2 diabetes have shown depressions in visual field with blue-on-yellow perimetry but not with white-on-white perimetry (Nitta et al., 2006). However, the reduced blue-yellow sensitivities could also be due to yellowing of the crystalline lens. Also a recent study by Park et al (2011) showed that mean deviation values (representing the overall depression or sinking in the visual field) worsened with increasing severity of nonproliferative DR. The finding was

similar to that observed by Takahashi et al (2006) in the overall defect and pattern defect (indicates local defects) in people without DR (Takahashi, et al., 2006).

However researchers have used modified or alternate visual field techniques such as flicker perimerty; for instance, authors have successfully demonstrated depression in visual field sensitivity within 6˚ from fixation using flicker perimetry in individuals with type 2 diabetes with no or minimal DR and also for those with duration of diabetes ≤ 5yrs (Stavrou et al., 2005). The rationale was that identification of flicker requires greater metabolic demand and therefore can detect visual field defects despite the absence of visible signs of DR (Lott et al., 2013; Scheiner et al., 1994). With the use of red-on-white perimetry, significantly greater visual field defects were demonstrated in patients with diabetes and because of the longer wavelength, it is not affected by the cataractous lens (Zele et al., 2008).

Standard automated perimetry (SAP) has potential limitations in that it is a subjective technique. In addition, it has been demonstrated that there is already 30-40% of retinal nerve fibre loss before any obvious field defect appears on standard perimetry (Quigley et al., 1982). With the advancement in imaging techniques, clinicians are now able to objectively assess the early changes in the human visual system. OCT has been demonstrated to have the ability to detect defects in the retinal nerve fibre layer when the standard automated perimetry results are normal (Kim et al., 2007). The current research program has utilized OCT for structural evaluation of the retina in diabetic participants.

Frequency doubling perimetry

Frequency doubling perimetry (FDP) was initially developed to test for the function of a subtype of ganglion cells. Realini et al (2004) observed diabetes to confound FDP results while screening for glaucoma. Participants in the diabetes group had higher test scores and took longer time than those without diabetes. However, impaired contrast sensitivity in diabetes may be an explanation for

the abnormal perimetry results (Realini et al., 2004)irrespective of neuronal loss in diabetes.

Electroretinogram and visually evoked potentials

Studies that measured the retinal electrophysiological responses observed abnormalities despite the absence of clinical signs of DR. Individuals with diabetes had reduced oscillatory potential amplitudes and delayed latencies (Barber, 2003), suggesting that the middle and inner retinal layers and possibly amacrine cells are susceptible to vascular insult even in the absence of signs of DR. The P100 latency of visual evoked potentials (representing electrophysiological responses of the nervous system and hence visual function) (Tobimatsu et al., 1991) was delayed in young people with diabetes without DR, which normalized following optimal metabolic control (Verrotti et al., 2000). Another study showed pattern ERG responses (a measure of central retinal function as well as ganglion cell function) were affected in glaucoma suspects with diabetes without DR as compared to glaucoma suspects without diabetes (Ventura et al., 2010). This could mean early changes in the inner retinal layers in diabetes despite the absence of clinical signs of retinopathy. These results indicate neuroretinal changes in the absence of visible vascular changes in the eye and can also suggest that the quality of some aspects of vision may become compromised in these eyes. Evaluating the structural integrity of the retina with imaging techniques will provide additional knowledge about the early changes in the retinal layers in diabetes. The current thesis examined the structural integrity in terms of retinal tissue thickness, in the presence and absence of clinical signs of retinopathy.

Full retinal thickness in the absence of retinopathy

The first part of this section provides a review of those studies that analysed the foveal thickness in diabetic people, in the absence of DR. Foveal thickness has been reported to increase with age in individuals with diabetes (Sánchez-Tocino et al., 2002, Fritsche et al., 2002, Kashani et al., 2010, Ooto et al., 2011) when compared to individuals without diabetes. However, there were differences in

mean age of individuals between the two groups; a mean difference of 4 years in the Sánchez-Tocino study and about 29 years older in the Fritsche study. This difference in age may have confounded the results, as the RPE metabolism has been reported to slow down with time. As a result, the degenerated cells and the outer segments of photoreceptors may accumulate over time (Ooto, et al., 2011). This phenomenon might be exaggerated in diabetes. In contrast, another study found no significant differences in the thickness within the central 1mm zone between individuals without DR and those without diabetes (Alkuraya et al., 2005).

Few other studies (described below) examined retinal thickness in the macular area. The retinal thickness in the parafovea (region just outside the fovea) was decreased in 29% of people with diabetes who also had subnormal Rarebit fovea test (Nilsson et al., 2007). Rarebit fovea test is reportedly used for testing of foveal function. Lower macular thickness has been reported in people with diabetes without DR compared to those without diabetes (Lattanzio, et al., 2002) (Oshitari, et al., 2009) and a trend of increasing thickness with increasing severity of DR was observed. In contrast, Sugimoto et al (2005) observed thicker retina in all quadrants with significantly thicker superior quadrant in people without DR than those without diabetes.

Nevertheless, the following studies reported no difference in macular thickness between the groups with and without diabetes, but observed that sex of the individual can be a factor related to retinal thickness. A group of authors observed no significant differences in retinal thickness in people with diabetes without DR when compared to those without diabetes, after the groups were matched for age and sex (Biallosterski et al., 2007) (Bressler et al., 2008). Browning et al (2007) reported macular volume data in addition to macular thicknesses for those without diabetes, individuals with diabetes but without DR, and other stages of DR. In normal eyes as well as in eyes without DR, the central subfield mean thickness (CSMT) was thicker and total macular volume was greater in men than in women. The mean between-gender difference was 14 μm in people without diabetes and 12 μm in eyes without DR (Browning et al., 2008). Oshitari et al (2009) observed that men in the 'no DR' group had

significantly thicker macular quadrants than women without DR. This suggests that sex of the individuals should be taken into account when examining retinal thickness in diabetes.

Other studies observed compromised retinal thickness in individuals with longer duration of diabetes (Bressler et al., 2008, van Dijk et al., 2010) but observed no significant association with HbA_{1c} levels (Asefzadeh et al., 2008).

The inconsistencies in study results could be due to differences in age, sampling error, differences in male and female composition or the extent to which individual retinal layers are affected in diabetes. The collective evidence from previous studies indicates that DR, age, gender and duration of diabetes need to be taken into consideration while examining the retinal tissue thickness.

Inner retinal thickness in the absence of retinopathy

RNFL thickness has been documented to be reduced in people with diabetes without DR compared to those without diabetes, with scanning laser polarimetry, (Ozdek et al., 2002; Takahashi, et al., 2006). Nonetheless, it can be argued that scanning laser polarimetry gives only a measure of birefringence rather than the actual thickness profile. Recent versions of OCT with much higher resolution should provide a better understanding of the specific retinal layers affected in diabetes.

The inner retinal thickness has been investigated in the following studies in people with diabetes in the absence of DR using OCT. Sugimoto et al (2005) observed slightly decreased superior quadrant RNFL thickness in a cohort of people without DR compared to those without diabetes. Another study found no significant differences in RNFL thickness between people with diabetes without DR and those without diabetes but observed significantly reduced superior GCC thickness (Park, et al., 2011). Recently, Verma et al (2012) demonstrated significantly decreased mean RNFL thickness in people with diabetes without clinical evidence of retinopathy compared to those without diabetes (Verma et al., 2012). Peng et al (2009) also observed reduced RNFL thicknesses in the superior and inferior sectors (between-group difference of 8 μm) and in the

mean RNFL (mean difference of 4 μm) between those with and without diabetes (Peng et al., 2009). On the other hand, two other studies observed no significant differences in RNFL or GCC thicknesses between the group of people without DR and those without diabetes (van Dijk, et al., 2009) (Oshitari et al., 2009).

Recent investigators observed reduced inferior RNFL thickness to be related to increasing severity of neuropathy in people with type 2 diabetes that was not explained by DR (Shahidi et al., 2012). This finding introduces the novel concept that reduced neural layer thickness in people with diabetes is explained by neuropathy rather than retinopathy.

Other variables such as HbA_{1c} levels and duration of diabetes and their relationship to inner retinal thickness have been investigated. Significant reduction in the RNFL thickness has been observed after 4 months of strict control of HbA_{1c} levels (Sugimoto et al., 2010) suggesting a possible association with HbA_{1c} levels. However, GCC thickness was not significantly explained by HbA_{1c} levels when adjusted for age, sex, duration of diabetes and retinopathy (van Dijk et al., 2010).

The inconsistencies in study results could be due to sampling error, the extent to which individual retinal layers are involved in diabetes or related to the presence or absence of neuropathy or in relation to glycaemic levels. Though there are differences in study results, it is likely that inner retinal thickness is reduced in the absence of retinopathy. To summarize, the full retinal and the inner retinal thickness is reduced in both, the presence and the absence of clinical signs of DR. In addition, factors such as duration of diabetes and HbA_{1c} levels have to be taken into account while examining the full retinal and inner retinal thicknesses.

This thesis presents a series of cross-sectional studies that investigated the full retinal and inner retinal thicknesses in individuals with diabetes, with and without neuropathy, adjusting for the known confounding factors such as DR, age, sex, duration of diabetes and HbA_{1c} levels. Examining the full retinal and neural layer tissue thickness in relation to diabetic neuropathy is the main

objective of this research program. General methodology for these experiments is described in Chapter 3 and methodology specific to the experiment addressing each research question is described in subsequent chapters.

Chapter 3: Research program

Summary of knowledge gaps

Early studies (described in Chapter 2. section 2.5.6) attributed the compromise in full retinal and inner retinal thickness as being solely related to DR. Further studies observed compromised full retinal and inner retinal thicknesses even in the absence of clinical signs of DR suggesting that there might be another factor that explains this degeneration.

1) Recent studies (elaborated in Chapter 1 and summarized below) reported that the neuroretinal layer thickness is significantly reduced in individuals with diabetes that was explained by peripheral neuropathy rather than DR (Shahidi et al., 2012). Therefore, it is likely that the retinal structural and visual functional compromise reported in literature may be confounded by diabetic peripheral neuropathy and therefore warrants investigation.

2) It is reported that neuroretinal thickness (Shahidi et al (2012), retinal function (Lövestam-Adrian et al., 2012), visual field sensitivity (Sampson et al., 2012) are compromised in relation to neuropathy. If RNFL thickness is compromised, it is likely that other retinal layers may be involved in diabetic peripheral neuropathy. In addition, if RNFL thickness is reduced in relation to neuropathy, GCC thickness may be reduced as well. It has been stated that the ganglion cell layer thickness can independently predict the visual function (van Dijk et al., 2011). Therefore, the integrity of other retinal layers, including that of the ganglion cell complex, requires investigation in relation to neuropathy.

3) The integrity of the macula in relation to diabetic neuropathy is not known. As the macular and foveal integrity are crucial for visual function, the integrity of the macula needs to be examined in relation to diabetic neuropathy.

4) Several independent studies demonstrate that retinal tissue thickness is influenced by a range of factors, namely age, sex of the individuals, ethnicity,

duration of diabetes, HbA_{1c} levels and DR. However, a 'full model' approach to account for these potential confounders has not been explored previously.

One way to analyse these knowledge gaps is to determine the relationship between retinal tissue thickness and diabetic peripheral neuropathy, adjusting for the confounding variables.

Therefore, the study by Shahidi et al (2012) was chosen as a foundation upon which further research questions were developed and explored in a comprehensive manner.

Objective of this research program

To determine the relationship between retinal tissue thickness and diabetic peripheral neuropathy

Hypothesis

It was hypothesized that the retinal tissue thickness is compromised in relation to diabetic peripheral neuropathy.

The current study performed a comprehensive evaluation of the retinal tissue thickness parameters, namely:

1) Full retinal thickness
2) Macular and peripapillary inner retinal thickness assessed in terms of
    a. RNFL thickness
    b. GCC thickness and pattern-based GCC parameters,

in a group with type 1 or type 2 diabetes and in those without diabetes or neuropathy (controls).

Key confounding variables that were taken into account include:

- Diabetic retinopathy (indicating clinically visible signs of retinopathy)
- Age
- Sex
- Duration of diabetes
- HbA_{1c} levels

The hypothesis was tested by a series of research questions in separate experiments.

Research questions

1. *Is there is a relationship between the type of diabetes and retinal tissue thickness?*
2. *Are the full retinal thickness and inner retinal thickness significantly related to the severity of diabetic neuropathy?*
3. *Are the focal and global losses in GCC volume significantly related to diabetic peripheral neuropathy?*

Further explanation for the research questions are provided in respective chapters.

It was expected that this research will provide new knowledge regarding retinal structural integrity in relation to diabetic peripheral neuropathy and will improve our understanding of the precise nature of retinal anatomical changes in diabetes, and how these relate to neural damage elsewhere in the body. This chapter describes the rationale for this research, followed by general methodology. The chapter discusses statistical analyses used in this research program. A more detailed discussion has been provided in subsequent chapters.

3.1 Rationale

3.1.1 Rationale for including people with and without diabetic neuropathy

Previous studies (referenced in Chapter 1, section 1.6) have demonstrated alterations to corneal nerve parameters in relation to diabetic neuropathy. Neuropathy has been proposed to be a microvascular complication (Malik et al., 1995) and as a neural complication (Tomlinson, 2002) of diabetes. The observation that corneal nerves are affected in neuropathy, given that the cornea is avascular, provides an interesting dimension to this argument. The theory that neuropathy is a vascular complication of diabetes, may be subject to debate in view of the corneal nerve deficits. However, a likely proposition for corneal neuropathy as a microvascular complication in diabetes could be that the cornea is also nourished by the aqueous (Pirie et al., 1956) which derives its nutrients from the blood supply to the ciliary body. If the vessels supplying the ciliary body are compromised in diabetic neuropathy, the corneal nerves may not be properly nourished and hence may be subject to insult. Another explanation could be that the pathogenesis in diabetes and hence neuropathy involves several theories, of which, ischemia (the obvious vascular component) is only one of them. The retina being a highly vascularised structure encompasses a wide range of neural components that play a vital role in vision; it is therefore crucial to evaluate the retinal integrity in relation to diabetic peripheral neuropathy. It is to be noted that the retinal nerve fibre layer thickness is reduced in association with diabetic neuropathy but not with retinopathy (Shahidi et al., 2012). In addition, literature that investigated retinal thickness in people with diabetes reported varying results. However, neuropathy status of the participants is not reported in those studies. Hence, there is a possibility that retinal layer changes observed in people with diabetes may be related to the presence of peripheral neuropathy. Therefore, this research project examined the full retinal and neural layer thickness in relation to diabetic neuropathy.

3.1.2 Rationale for examining participants with and without diabetic retinopathy

Diabetic retinopathy has been recognised as a putative mechanism behind retinal tissue degeneration until few other studies reported retinal tissue compromise even in the absence of DR, suggesting the likelihood of another factor that may be related to the compromised retinal tissue thickness in diabetes.

Recent studies have demonstrated reduced RNFL thickness (Shahidi et al., 2012), decreased visual field sensitivity (Sampson et al., 2012) and compromised retinal ganglion cell function (Lövestam-Adrian et al., 2012) in relation to neuropathy rather than retinopathy, suggesting that neuropathy is a factor that may be more important than DR when assessing neural layer thickness, optic nerve function and mid-peripheral visual field sensitivity in people with diabetes. This could possibly suggest a local response of the generalised polyneuropathy rather than DR that may be exerting subtle neural changes in the eye. Therefore, participants with retinopathy were not specifically excluded but retinopathy status was accounted for in the statistical models, along with other factors namely age, HbA_{1c} levels, duration of diabetes and sex of the individuals.

Due to the prolonged nature of diabetes, it is also reasonable to expect that people with DR may have co-existing DPN or vice versa. If people with DR were excluded, people with co-existing DPN may also be excluded. Consequently, the cohort may not truly represent people with diabetic neuropathy. Therefore, people with DR were not specifically excluded.

3.1.3 Rationale for examining full retinal thickness, RNFL and GCC thicknesses

The reason that nearly one third of all the neurons that converge at the optic nerve originate from the macular area, suggests that, macular integrity is an important predictor of visual function (Apple, 1981). The macula has the highest density of cones and therefore has an increased metabolic demand

(Antonetti et al., 2006); the macular area may therefore be susceptible to microvascular or ischemic events. As a result, the integrity of macula may be challenged in diabetes. Therefore, this research project was designed to examine the structural integrity of the retina in terms of full retinal thickness and GCC thickness in the macular area.

In addition, RNFL and GCC thicknesses have been studied extensively in the field of glaucoma for several years. Glaucoma is a disease process characterised by ganglion cell death and typical changes in the optic nerve head (Quigley, et al., 1982). For the reason that the loss of neuroretinal cells is irreversible, focus has shifted to early detection and prevention of further damage. However, ganglion cell death may not be limited only to glaucoma (Quigley et al., 1995). Recently, researchers have observed compromise to peri-papillary neural layers in relation to diabetic peripheral neuropathy (as in Chapter 1, section 1.6). For the reason that ganglion cells are several layers thick in the macular area, loss of ganglion cells may be easier to detect in this area (Zeimer et al., 1998). However, to date, there is no literature pertaining to full retinal or ganglion cell complex thickness in the macular area in relation to diabetic peripheral neuropathy. Therefore, this research project investigated the full retinal thickness, GCC thickness at the macular area and in addition, the peri-papillary nerve fibre layer thickness in people with diabetes, with and without neuropathy.

3.2 General methodology and research plan

3.2.1 Participants

Participants were recruited as a part of the LANDMark study conducted at Queensland University of Technology (QUT), Brisbane. Ethical clearances were obtained from QUT and Princess Alexandra Hospital Human Research Ethics Committees (Ref 0800000167 and 2008/058, respectively) (Appendix 1). Participants were recruited from Princess Alexandra Hospital and the broader Brisbane community by advertisements in magazines and newspapers. Participants provided written informed consent (Appendix 2).

The LANDMark study protocol

The Longitudinal Assessment of Neuropathy in Diabetes using novel ophthalmic Markers (LANDMark) study is a 4-year, two-site study that is aimed at the identification and longitudinal assessment of ophthalmic markers for diabetic peripheral neuropathy. The LANDMark study enrolled a cohort comprising of 315 participants at baseline visit; groups of individuals with diabetic neuropathy, without neuropathy and controls, were examined at baseline and are followed up annually for 4 consecutive years. The study is ongoing, and is not expected to be completed until mid-2015.

As a part of the LANDMark study research protocol, a large number of tests are being conducted. Several optometrists are involved in the ophthalmic examination of the study participants. A range of tests conducted as per the protocol are as follows:

1) Detailed ophthalmic history
2) Visual acuity assessment
3) Slit lamp examination of the eye to assess ocular status of the participants
4) Non-contact corneal aesthesiometry
5) Corneal confocal microscopy
6) Visual fields examination
    a. Medmont automated perimetry
    b. Frequency doubling perimetry (performed twice)
7) Optical coherence tomography (acquisition of RNFL, GCC, macula and ONH scans)
8) Fundus photography
    a. Colour
    b. Red-free
9) Three consecutive intra ocular pressure measurements
10) Slit lamp examination at the end of the examination
11) Uploading the data on to the research group database
12) Preparation of health care practitioner reports as needed

13) Follow-up calls to the participant the day after the visit to thank participants and seek assurance of no ill-effects of study participation.

A more detailed description of the LANDMark study design as well as the baseline characteristic of participants with type 1 diabetes has been described elsewhere (Pritchard et al., in press).

The work described in this thesis analyses a subset of the LANDMark dataset at the Brisbane site to address specific hypotheses relating to retinal thickness changes associated with DPN.

Methodology specific to this research program of the candidate

This research project comprised of a series of cross-sectional, observational studies to analyse the research questions. The presence and type of diabetes was by self-report or ascertained from their health care provider. Individuals identifying as 'without diabetes', had fasting plasma glucose level in the normal range per our local pathology provider. Anyone with a failed fasting plasma test underwent an oral glucose tolerance test; although the protocol allowed impaired fasting glucose, all individuals had fasting glucose in the normal range. Information about duration of diabetes and ethnicity was self-reported. Glycosylated haemoglobin level (HbA_{1c}) was assessed on the day of the examination. Individuals underwent neuropathy evaluation (explained below) and were classified as with or without neuropathy according to NDS criteria. Medical and demographic data were collected by qualified and experienced personnel.

Participants were chosen based on the following inclusion and exclusion criteria.

Inclusion criteria specific to this research program

- Aged 40 years and above
- Type 1 or type 2 diabetes, and no diabetes or neuropathy for control group
- Best-corrected visual acuity equal to or better than 6/9
- Spherical refractive error within ± 6 D, astigmatism within ± 3 D
- Any stage of diabetic retinopathy defined using ETDRS scale

Exclusion criteria specific to this research program

- Coexisting ocular infection or inflammation
- IOP > 22 mmHg
- Individuals with a vertical and horizontal cup-disc ratio > 0.6
- Cataract that prevents good view of posterior segment in fundus photography or conduct of OCT
- History of systemic disease (e.g. malignant disease, diseases affecting optic nerve, multiple sclerosis, Parkinson's disease, that may affect the RNFL thickness)
- History of neuropathy due to non-diabetic cause
- Participating in any other interventional research trial

For the control group, in addition to the above criteria, participants were excluded if they had any of the following criteria:

Additional exclusion criteria for the control group

- Diabetes
- GADab (Anti-glutamic acid decarboxylase antibody) positive
- Any neuropathy

Sample size calculation for the current research program

As a preliminary step, the differences between the groups were analysed in the key variables and then in the retinal tissue thickness. Finally, the relationship between retinal tissue thickness and diabetic peripheral neuropathy was analysed. The GPower 3 software (Faul, Erdfelder, Lang & Buchner, 2007) was used to calculate the minimum difference to be detected between the groups: diabetes without neuropathy and diabetes with neuropathy. The desired power and significance level were set at 0.80 and 0.05, respectively and a priori analysis (sample size N has been computed as a function of power level 1-β, significance level α, and the effect size) was applied. For regression analysis, the R squared deviation from zero was calculated. The hypothesis for each experiment is discussed in the respective sections of experiment.

Sample size calculation for full retinal thickness

To address the research question,

Is the full retinal thickness in people with neuropathy different from those without neuropathy?

For a mean difference of 10 microns with a SD of 17 microns (Sull et al., 2010), it was estimated that 47 in neuropathy group, 47 in the no neuropathy group and 47 participants without diabetes or neuropathy (controls) will be required. Allowing 10% of drop-out rate, a total of 155 participants will be required.

Sample size calculation for inner retinal thickness

To address the research question,

Are the RNFL and GCC thickness in people with neuropathy different from those without neuropathy?

RNFL thickness

For a mean difference of 5 microns with a SD of 10 microns (Shahidi et al., 2012), it was estimated that 71 in neuropathy group, 71 in the no neuropathy group and 71 in the control group will be required. Allowing 10% of drop-out rate, a total of 234 participants will be required.

GCC thickness

For a mean difference of 5 microns with a SD of 7 microns (Garas et al., 2012), it was estimated that 52 in neuropathy group, 52 in the no neuropathy group and 52 in the control group will be required. Allowing 10% of drop-out rate, a total of 170 participants will be required.

Sample size for regression analysis using deviation from R squared

In a previous pilot study (Shahidi et al., 2012), with the RNFL thickness as the dependent variable, R squared was found to be 0.1 with 5 predictors in the model that analysed 82 individuals with type 2 diabetes.

Research question

Is there a relation between retinal tissue thickness (full retinal and inner retinal) and diabetic neuropathy?

No previous data exists in relation to full retinal thickness. For the current research program, it was estimated that a total of eight predictors will be included in the model; the regression models will have four continuous independent variables and two categorical independent variables with two levels in each; a sample size of 69 is required for an R squared of 0.2 and a sample of 150 for an R squared of 0.1. In other words, a sample size between 70 and 150 is required for an R squared of 0.1-0.2 at 80% power, α=0.05.

Table 2 provides a summary of the parameters for the sample size calculation for the full retinal and inner retinal thicknesses.

Table 2. Sample size parameters calculated using GPower software

A priori calculation	Full retinal thickness	RNFL thickness	GCC thickness	Retinal tissue thickness Regression: R^2 deviation from zero
Input: Tail(s)	Two	Two	Two	
Effect size d	0.5882	0.4756	0.5555	$f^2 = 0.1111$
α err prob	0.05	0.05	0.05	0.05
Power (1-β err prob)	0.8	0.8	0.8	0.8
Allocation ratio N2/N1	1	1	1	Number of predictors = 8-9
Noncentrality parameter δ	2.8515	2.8340	2.8327	λ = 15.9999
Critical t	1.9860	1.9770	1.9834	F = 2.0076
Df	92	140	102	Numerator df = 8
Sample size group 1	47	71	52	Denominator df = 135
Sample size group 2	47	71	52	
Actual power	0.8055	0.8036	0.8012	0.8030
Total sample size	141	213	156	144

The sample size for the RNFL thickness was the highest. Therefore this was taken as the minimum sample size required for the entire research program.

The following sections discuss the neuropathy and the ophthalmic tests that were performed.

3.2.2 Neuropathy assessment

In the current research program, neuropathy was defined using the NDS criteria. Individuals with and without diabetes also underwent comprehensive assessment for neuropathy. The following section provides a brief review of the tests used in the detection of diabetic peripheral neuropathy (as previously explained in detail in Chapter 1, section 1.5).

3.2.2.1 Neuropathy disability score (NDS)

The test involved neurological examination of three sensory modalities: vibration perception with a 128-Hz tuning fork on the great toe, sharp and blunt sensation using a Neurotip® device (Owen Mumford Ltd., Oxford, UK) on the plantar aspect of the great toe; and hot and cold temperature sensation was assessed on the arch of both feet using metal rods immersed in hot and cold water. A score of 0 was given for normal response and 1 for abnormal response for each individual test component.

Additionally, the ankle reflex was assessed using a reflex hammer with the scores, 0 for normal, 1 for reinforcement and 2 for absent. Each foot can have maximum score of 5 resulting in a total score of 10 for both feet. An NDS criterion of neuropathy was defined as NDS score ≥3 on a scale of 10 (Young et al., 1993), with higher scores indicating more advanced degrees of neuropathy.

3.2.2.2 Diabetic neuropathy symptom score (DNSS)

This is a questionnaire that contains four general questions regarding unsteadiness while walking, experience of burning, aching pain or tenderness in legs or feet, prickling sensation in legs or feet and places of numbness in legs or feet. A score of 1 was given for the presence of symptom and 0 for the absence of symptom. A score of 1 or more out of 4 was recorded as abnormal DNS score (Meijer, et al., 2002).

3.2.2.3Nerve conduction/Electrophysiology

Assessments were done on the peroneal, tibial or sural nerves in the lower limb on the hand-dominant side of the participants using the Nihon Kohden Neuropack S1; MS92a EMG (Medelec Limited, Old Working, Surrey UK). Nerve conduction velocities and amplitudes were compared with the age-related normal values (provided in Table 3). Peroneal nerve conduction velocities and amplitudes were analysed for all participants as the peroneal nerve parameters were recordable for almost all participants. Sural nerve measurements were obtainable in a minority of individuals, and are not reported here.

Table 3 provides a summary of age-based normal values of conduction velocities according to LANDMark study in-house norms.

Table 3. Criteria for abnormal nerve conduction velocities based on the age

	Age < 54 years	Age ≥ 54 years
Abnormal tibial conduction velocity	< 43 m/s	< 43 m/s
Abnormal peroneal conduction velocity ankle to fibular head	< 45 m/s	< 42 m/s
Abnormal sural conduction velocity calf to ankle	< 40 m/s	< 38 m/s

a. < 10th percentile for healthy individuals without neuropathy at Brisbane site.
b. Nerve conduction (NC) is considered abnormal if *either* peroneal or sural nerve conduction velocity (NCV) is below age-reference cut-off values.
c. If sural not present, NCV is considered abnormal if tibial NCV is below 43 m/s for any age.

3.2.2.4Quantitative sensory testing

The thresholds for vibration, thermal and pain stimuli such as heat-induced and cold-induced pain were recorded and analysed. The Medoc Quantitative Sensory Analyser, Model TSA-II (Medoc Advanced Medical Systems, Ramat Yishai 30095, Israel) was utilized.

3.2.2.5 Monofilament test

The 10g nylon monofilament test was utilized to assess touch perception by exerting a specific repeatable bending force on three test sites; the participant responded whether they felt it or not. The number of points felt out of three was recorded.

3.2.3 Ophthalmic assessment

The eye on the side of the dominant hand was tested unless contraindicated by the exclusion criteria, in which case, the eye on the non-dominant side was tested. Participants underwent visual acuity assessment, slit lamp examination, intraocular pressure measurement and pupil dilation after confirming adequate anterior chamber depth by the van Herick method. Three-field fundus photographs were taken for colour and red-free evaluations (section 3.2.3.1). The full retinal and the inner retinal tissue thicknesses for RNFL around ONH and GCC thicknesses at macula were measured using spectral domain OCT (section 3.2.3.2). People who had undergone laser treatment were not included in the main experiments detailed in Chapters 4-5. However, the retinal tissue thickness in people who had undergone laser for DR has been examined and is discussed in Chapter 6.

3.2.3.1Fundus photography

Three-field photographs were obtained, each 45 degrees, covering nasal, central and temporal retinal regions (Visucam Pro, Carl Zeiss Meditec Inc., Dublin, CA, USA). Diabetic retinopathy grading was performed using the Early Treatment Diabetic Retinopathy Study scale (ETDRS) by an Ophthalmologist who was masked to clinical information of the participants.

3.2.3.2Spectral domain optical coherence tomography

Retinal evaluation in diabetes has been conventionally done with ophthalmoscopy, slit-lamp biomicroscopy, and sometimes with fundus

fluorescein angiography. Early detection of changes in ocular health has become essential for timely referral in order to initiate treatment.

Imaging technologies have provided the gateway to assess structural changes in the ocular tissue. Optical coherence tomography (OCT) has become an indispensable technology for the clinicians to assess subtle changes in the retina. The spectral domain OCT (SD-OCT) aids in non-contact evaluation of retinal layers in-vivo at a near-microscopic resolution; the OCT provides thickness information of the layer of interest and has been employed in a number of studies. The current research program utilized RTVue SD-OCT (Optovue, model RT-100, ver.4.0, Fremont, CA, USA) (Figure 16) for investigating retinal tissue thickness.

Figure 16. RTVue spectral domain OCT

The principle has been described elsewhere (Schuman, 2008); however a brief review is provided here. The OCT employs a wavelength of 820 ± 10 nm from a super-luminescent diode. The beam is split into a reference arm that is sent to a mirror and the other that is sent to the ocular tissue. The reflected beam from the ocular tissue and that from the mirror create interference pattern, which is split by a grating into different wavelength components. Fourier transformation is then applied to give A-scans. The software acquires 26,000 A-scans in one second with an axial resolution of 5 μm (Adapted from the RTVue User Manual, ver.4.0). The MM6, RNFL, and GCC scan protocols (described below) were used to obtain thickness information.

Full retinal thickness

The MM6 protocol of the RTVue OCT consists of 12 radial lines each 6mm long centred at the fovea to provide 1024 A-scans each. The full retinal thickness is reportedly measured from the vitreoretinal interface to the outer-inner segment layer of the photoreceptors (Figure 17) at three concentric zones: a circle of 1 mm diameter centred at the fovea, a parafoveal region that has inner diameter of 1mm and outer diameter of 3 mm and a perifoveal region that has an inner diameter of 3mm and outer diameter of 6 mm.

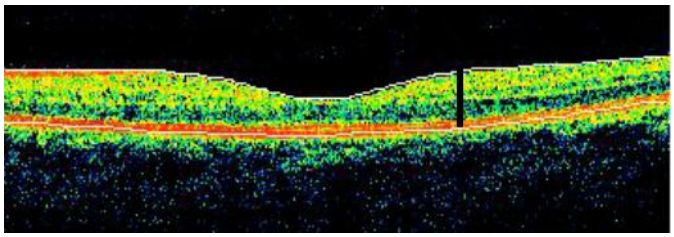


Figure 17. The retinal thickness is measured from the internal limiting membrane to the IS-OS junction of the photoreceptors (indicated by the black line)

Figures 18A and 18B illustrate the scanned retinal area and the three concentric zones: central zone, the parafovea and the perifovea (American Diabetes Association, 1985) respectively. In addition to the overall thicknesses, the hemisphere thicknesses in the parafovea, perifovea as well as for inner retinal thickness were analysed. Figure 19 shows the hemisphere regions analysed in the current project.

The rationale for examining as overall and as hemisphere thickness is that the inferior RNFL thickness (Shahidi et al., 2012), mfVEP amplitudes (Lövestam-Adrian et al., 2012) in the lower nasal retinal quadrant, are compromised in relation to diabetic peripheral neuropathy. Also Harris et al (2003) reported that the blood flow per nerve fibre tissue volume may be reduced in the inferior retina compared to the superior retina and hence may be more vulnerable to vascular insults. Therefore, the current research project sought to examine retinal tissue thickness in the superior and inferior hemispheres as well apart from the overall thickness (Figure 19).

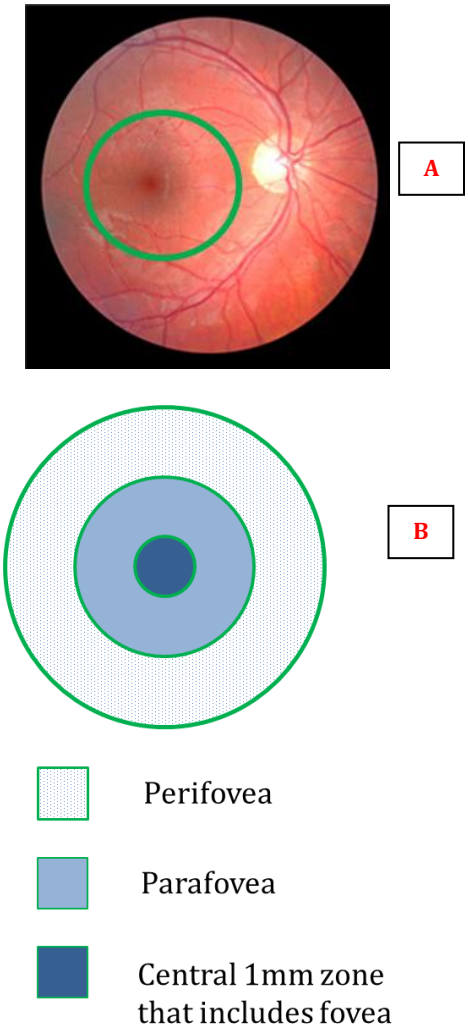


Figure 18. (A) The scanned area in the retina for retinal thickness (B) The three concentric zones, the central zone, the parafovea (inner macula) and the perifovea (outer macula)

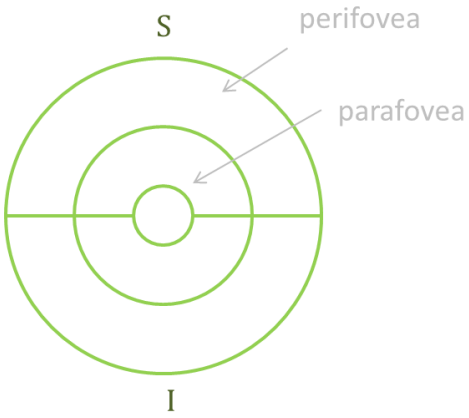


Figure 19. Retinal thickness in this project were further examined as overall thickness in the central zone, parafovea and perifovea as well as hemispheres of parafovea and perifovea

Repeatability of full retinal thickness measurements

The variability in retinal thickness measurements in healthy populations have been reported in the literature. For instance, the test-retest variability for macular thickness parameters has been reported to be between 2.8 μm and 4.5 μm for the inner macular quadrants and between 2.8 μm and 6.2 μm for the outer macular quadrants (Nakatani et al., 2011). The difference in retinal thickness between groups is expected to be greater than the repeatability of the measurements such that meaningful differences between groups can be elucidated.

Inner retinal thickness

Figure 20 is a representation of scanned areas for the RNFL and GCC thicknesses and their corresponding measured zones.

RNFL parameters

Peripapillary nerve fibre layer thickness (RNFL) is purportedly measured from the inner border of nerve fibre layer to outer border of the plexiform layer along a circle of 3.45 mm diameter centred at the optic nerve head. The macular inner retinal layer thickness is measured from inner plexiform layer to the nerve fibre layer at the macula.

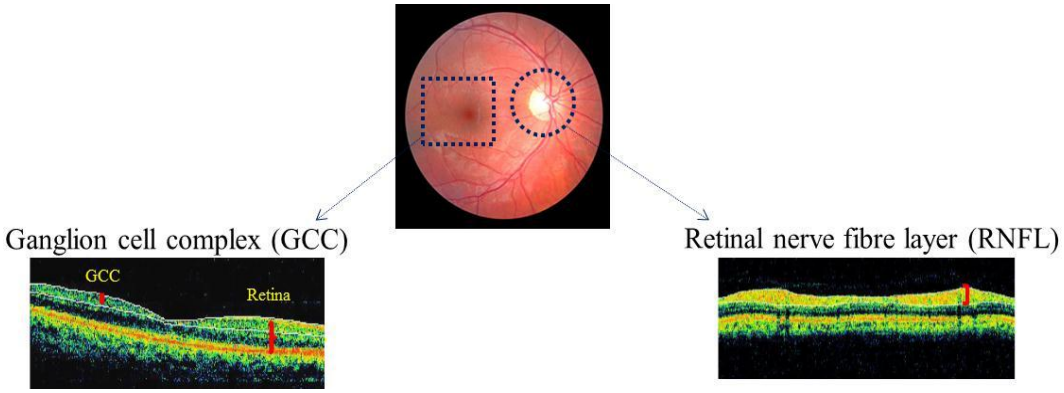


Figure 20. The top picture shows scanned areas for the inner retinal thickness. Bottom left picture shows the ganglion cell complex (GCC) scan. Bottom right picture shows retinal nerve fibre layer (RNFL) scan.

Retinal nerve fibre layer (RNFL) thickness around the ONH is provided as 16 sectors that are colour coded for rapid visual inspection in comparison to the thickness values from the normative database. Overall RNFL is the mean of RNFLT (µm) for the total 360˚ measuring circle of 3.45 mm diameter centred at the ONH. Superior average RNFLT (µm) is measured in the superior hemisphere of the measuring circle. Inferior average RNFLT (µm) is measured in the inferior hemisphere of the measuring circle (Figure 21).

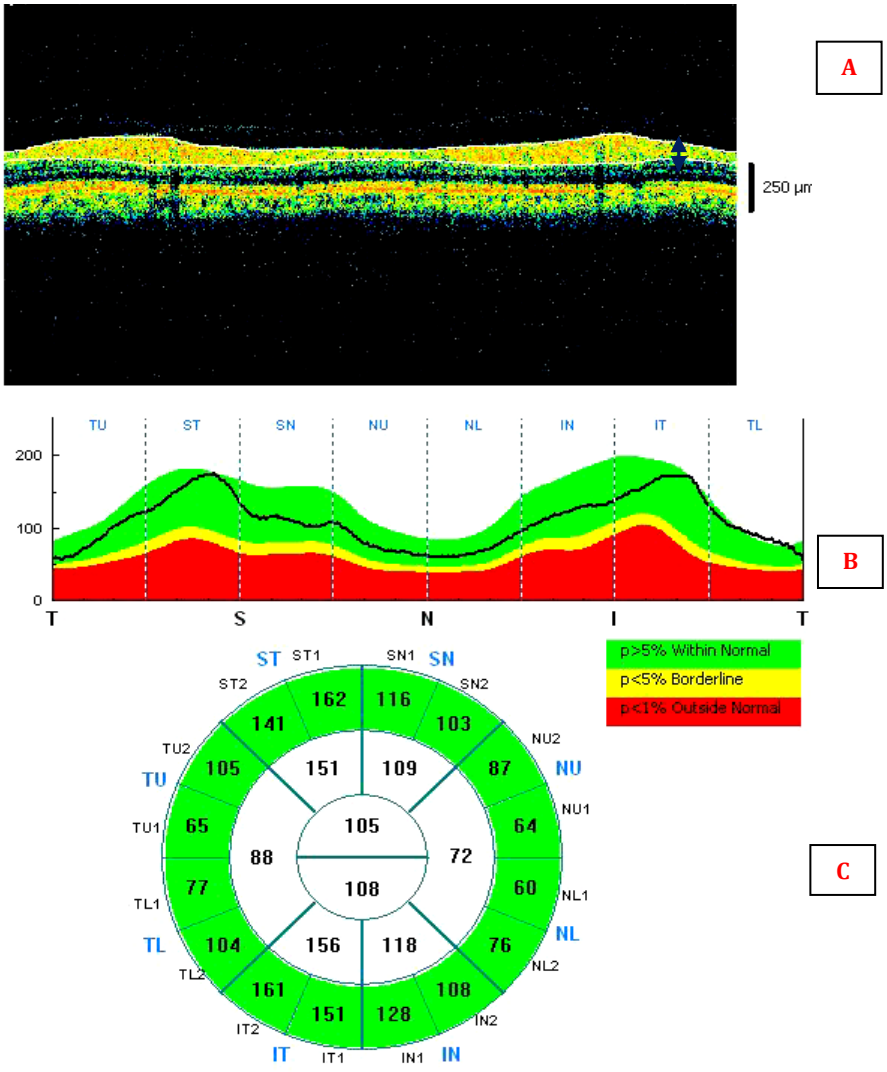


Figure 21. RNFL scan in RTVue SD-OCT (A) RNFL thickness for the individual shown by black arrow (B) TSNIT graph showing the average RNFL thickness matched against that from normative database (C) RNFL thickness divided into 16 sectors. For this research program, the overall RNFL and the averaged thicknesses in the superior and inferior hemispheres were analysed

GCC parameters

The ganglion cell complex (GCC) is a composite of retinal nerve fibre layer, ganglion cell layer and inner plexiform layer. The GCC protocol scans over a 7 mm x 7 mm zone with 15 vertical lines and one horizontal line centred at 1 mm temporal to fovea so as to cover the maximum of macular area. The protocol provides 15,000 A-scans within 0.6 seconds. The output includes thickness map and the significance map that is derived from the deviation map (Figure 22)

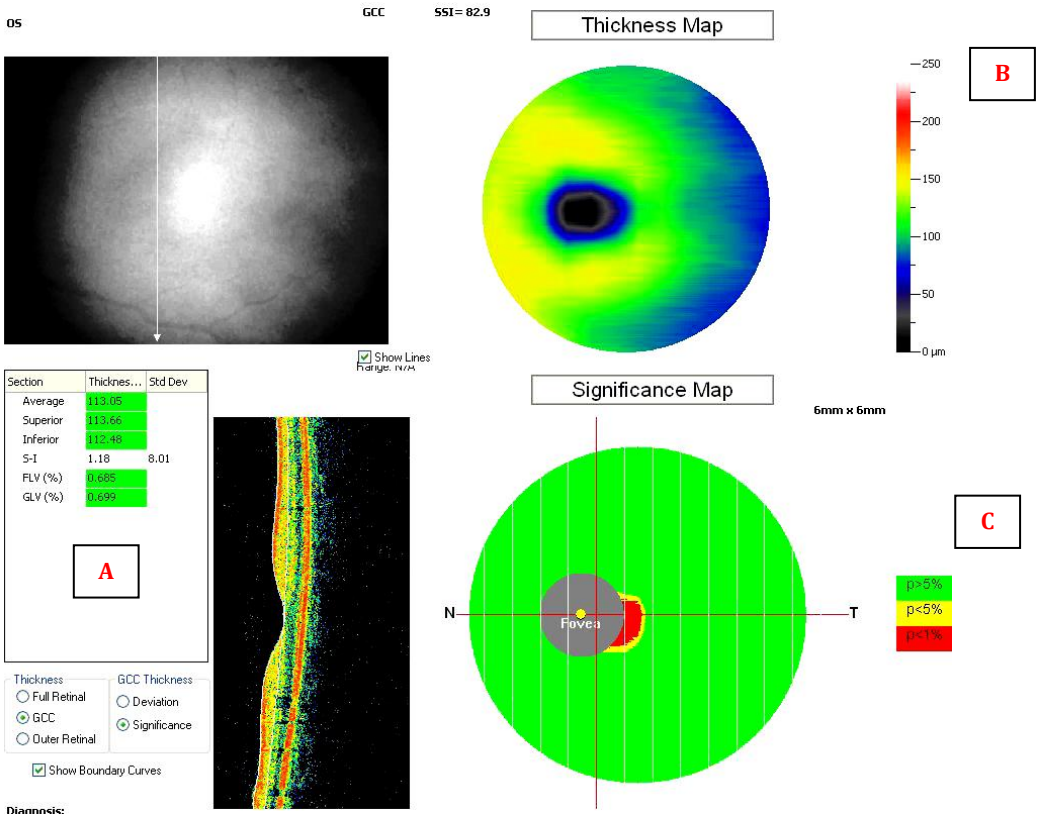


Figure 22. GCC scan in RTVue SD-OCT. (A) Summary of GCC parameter is given in the table on the left (B) Thickness map: warmer colours represent thicker regions and cooler colours represent thinner regions (C) Significance map at the bottom represents the significance of the difference in comparison to population norms

Overall GCC thickness reflects the average of the superior and inferior GCC values. Superior hemisphere GCC is the thickness of all layers between the nerve fibre layer and the inner plexiform layer in the area above the horizontal meridian. Inferior hemisphere refers to thickness of all layers between the nerve fibre layer and the inner plexiform layer in the area below the horizontal meridian.

Repeatability of measurements

Garas et al (2010) have shown that the intratest (i.e within a series of test measures taken on the same occasion) variability for average RNFL thickness is 2.91 μm and 3.45 μm and 3.11 μm for superior and inferior hemisphere GCC, respectively. The intertest (i.e between repeated test measures on different occasions) variability is 4.25 μm for the average RNFL and 4.51 μm and 3.93 μm for the superior and inferior GCC, respectively (Garas et al., 2010). Therefore, differences between the groups with and without neuropathy should be greater than the repeatability to be considered as meaningful differences between groups.

Pattern-based GCC parameters

The pattern-based GCC parameters include global loss volume (GLV), focal loss volume (FLV) and root mean square (RMS). These parameters have been summarised below.

GLV indicates the global loss in GCC volume over the entire GCC map. The percentage decrease in thickness at each pixel compared to the population norms is calculated. The resulting map is called the fractional deviation map where the pixels with values < 0 are summed up and divided by the entire GCC area to give GLV (%).

FLV indicates the focal loss in GCC volume over the entire GCC map. The thickness value at each pixel is compared to that of age-matched normative database from the machine to give a pattern map. This map is then compared with the average pattern map from the age-matched normative database. The difference between the two maps is the pattern deviation map, which is colour coded and assigned significance values for the deviation. The pixel values in the pattern deviation map which had values < 0 in fractional deviation map are summed with those for a p < 5% from pattern deviation map and divided by the total area to give FLV.

RMS or root mean square represents the coefficient of variation. It is an index to show how well the fractional and pattern deviation maps of an individual fit the normal pattern. The higher the number, the poorer is the fit. For this research project, the FLV and GLV were analysed.

Only well centered scans with signal strengths better than 35 were taken as 35 is the minimum signal strength required to register a scan (Balasubramanian et al., 2009). If the desired signal strength is not achieved, the particular scan protocols were repeated. One acceptable image with highest signal strength for each scan type was considered eligible for analyses.

Final sample size eligible

Figure 23 is a flow chart showing the number of participants in each sub-group. The eye on the hand-dominant side was chosen if ophthalmic data were eligible for that eye. If not, the other eye was included if eligible.

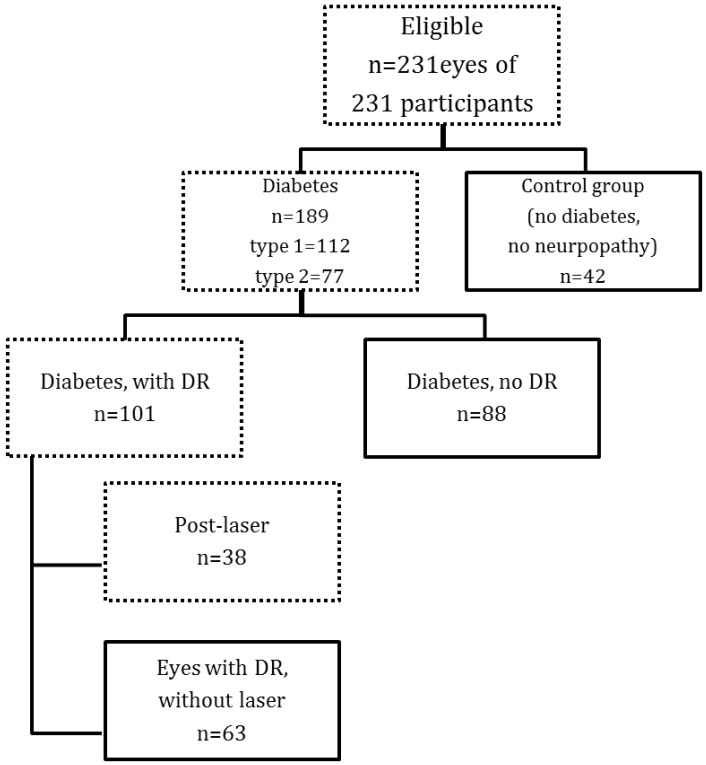


Figure 23. The number of participants in each group. DR, diabetic retinopathy. Solid boxes represent final number of participants who were eligible

Data extraction and handling

The retinal thickness data and fundus photographs were acquired from the OCT and the fundus camera respectively. The medical and neuropathy data were obtained from the online database of the research group where in, the data pertaining to medical, neuropathic and ophthalmic assessments is being routinely uploaded by registered nurses, electrophysiologist and optometrists.

The eligibility pertaining to visual acuity, intra ocular pressure and spectacle prescription was assessed with reference to the data folder of every participant.

Exporting OCT data

The eligible eye of the participants was recorded. The OCT data was exported in 'xml' format. Subsequently, the type of scan, for instance, 'RNFL 3.45' was chosen. The participants and their visits were then were chosen based on the date of examination. This procedure was repeated separately for GCC thickness data and the macular thickness data. The exported data was then converted as Microsoft Excel datasheet and saved under a filename.

Exported data was then reorganised in relation to specific retinal zones to facilitate subsequent data analysis.

Data pertaining to history, medical and neuropathy assessments, fundus grading, retinal thickness measures, visual acuity and intra ocular pressures were all combined into one master Microsoft Excel datasheet so that every participant had a single row of data. The data sheet was then scanned for missing data points. In case of data that were missing, the same was obtained by examining the data folder for every participant and re-entered into the master excel sheet.

Pilot study by Shahidi et al (2012)

The previous pilot study by Shahidi (2012) entitled "Retinal nerve fibre layer thinning associated with diabetic peripheral neuropathy", constituted a collaborative effort from the LANDMark laboratory. A detailed description has

been presented in section 1.6.2. However, the study examined individuals with type 2 diabetes and controls only. The RNFL thickness was the only outcome variable assessed in the study. The current research program has taken a more comprehensive approach and has examined full retinal thickness, the RNFL thickness, GCC thickness and the pattern-based GCC parameters in relation to diabetic peripheral neuropathy. Individuals with type 1 or type 2 diabetes were examined. In addition, a larger number of controls were examined.

Therefore, it is to be noted that there is a proportion of participants in the current project that were also included in the study presented by Shahidi et al (2012). The current study has used slightly different inclusion criteria. As a result, 67 individuals with type 2 diabetes in the current project represent about 90% of type 2 cohort included in Shahidi's study. In the control group, 57% of the individuals in the current project were a part of Shahidi's control group. The current project has included 84 individuals with type 1 diabetes, who have not been examined so far.

Contribution of the candidate towards the current research program and to the overall group research project

As a member of the LANDMark research team, the candidate has been actively involved in examining participants over the entire duration of her PhD candidature. The candidate has been involved in

(i) Examination of study participants, data collection, recording observations on the Case Report Forms.

The candidate has examined about 290 participants over the duration of the PhD candidature; this total number is inclusive of some participants who were examined twice or even thrice by the candidate during the subsequent years. For the set of procedures conducted as per the LANDMark study protocol, the examination time was approximately 2 hours per participant.

(ii) Data management and analysis (data export, uploading various data forms e.g. JPEG, mxf, pdfs to the study database).

(iii) Contributing to scientific discussion regarding study design, analysis and outcomes of all aspects of the LANDMark study.

(iv) Hospitality, e.g. greeting the participant, making them comfortable, to find their way in and out of study site, accompany the participants to the street and find them a cab.

(v) Courtesy telephone calls after 24 hour of examinations to thank the participant for their time and to ensure there were no ill-effects of participation. These details are recorded in the study database.

The involvement of the candidate in the data entry and management, preparation of referral letters and follow-up phone calls together constituted an additional 3 hours per participant-visit.

Of the total 231 participants included in the current study (as in Figure 23), 85% were examined by the candidate.

To address the research questions for the current research program, all data were included except the perimetry and corneal tests.

3.3 Statistical analyses

Statistical Package for Social Sciences (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp) was used for analyses.

A summary of the variables included in the current research program has been provided in Table 4.

Table 4. Variables used for statistical analysis

Variables	Variables of interest	Outcome variable for analysis
Retinal tissue thickness	Full retinal thickness (μm)	Continuous
	Inner retinal thickness (μm)	Continuous
	Focal and global loss in ganglion cell volume (expressed as percentage loss, %)	Continuous and Categorical
Neuropathy measures	Diabetic neuropathy symptom score	Continuous
	Neuropathy disability score	Continuous and Categorical
	Quantitative sensory testing: Temperature threshold: degree Celsius Vibration threshold: Hertz	Continuous
	Peroneal nerve amplitude (millivolts)	Continuous
	Peroneal nerve conduction velocity (meters/second)	Continuous
	10g monofilament	Continuous
Demographic and general health variables	Age (years)	Continuous
	HbA_{1c} (%), where, the HbA_{1c}/total haemoglobin ratio is expressed as percentage HbA_{1c}	Continuous
	Duration of diabetes (years)	Continuous
	Total cholesterol (mmol/L)	Continuous
	Systolic and diastolic blood pressure (mmHg)	Continuous
	Gender	Categorical
	Ethnicity	Categorical

As a preliminary step, the assumptions of normality and homogeneity of variance were tested before any group comparisons were performed. If the assumptions of normality or the homogeneity of variance was violated, then a non-parametric test was utilized for group comparisons. Normally distributed data for two-group comparisons were analysed using two-tailed unpaired t-tests. An analysis of variance (ANOVA) test was used for comparing three or more groups for normally distributed variables.

For instance, the group differences between type 1 diabetes, type 2 diabetes and the non-diabetic, no neuropathy group were analysed using ANOVA for normally distributed variables and with Kruskal-Wallis test for variables that did not follow normal distribution. For those variables where significant differences in means were noted with ANOVA, a post-hoc test was conducted using the Tukey HSD test. For the reason that there is a possibility of type I error due to multiple comparisons, a Bonferroni post-hoc test has also been performed to aid in the additional interpretation of the results.

To analyse relationships between variables, general linear models were applied. A general linear model can account for main effects, and interactions between variables. For analysing the effects of clinical and demographical variables on a categorical outcome variable (Chapter 5), binary logistic regression model was utilized. A p-value of < 0.05 was considered statistically significant.

In the entire thesis, the magnitude of the differences is discussed only for significantly different variables and their significance values are presented in the respective tables.

Chapter 4: Retinal tissue thickness and diabetic neuropathy

4.1 Age, sex and retinal tissue thickness in the control group

Chapter 2 discussed the literature from the general population demonstrating the relationship between retinal tissue thickness, age and sex of the individuals in the absence of diabetes. However, it is unclear if the presence of neuropathy was accounted for in those studies. In addition, there is discrepancy in literature regarding gender predilection for diabetic neuropathy. This is an important consideration for the reason that objective of this research program is to determine the relationship between retinal tissue thickness and diabetic neuropathy.

Therefore, it is essential to examine for the effects of age and sex on the retinal tissue thickness in its pure form: which means, in the absence of diabetes and in the absence of neuropathy due to non-diabetic causes. Therefore, as a preliminary step, the first part of the study investigated the relative effects of age and sex on the full retinal and inner retinal thickness in individuals who do not have diabetes or neuropathy due to non-diabetic causes. This section 4.1 presents the analyses in this group (control group).

4.1.1 Purpose

To explore the relationship between age, sex of the individuals and retinal tissue thickness in those without diabetes and without neuropathy

4.1.2 Research question and hypothesis

Is the retinal tissue thickness influenced by age and/or sex in individuals without diabetes or neuropathy?

It was hypothesized that age and sex of the individuals are significantly related to the retinal tissue thickness in those without diabetes and without neuropathy.

4.1.3 Methods

Participants who did not have diabetes were invited to participate in the study as previously described in Chapter 3. Individuals underwent a comprehensive ophthalmic, medical and neuropathy assessments to rule out diabetes as well as neuropathy.

Forty-two individuals met the criteria. The full retinal and inner retinal thicknesses were examined.

4.1.4 Statistical analysis

The variables age and retinal thickness parameters were examined for normality of distribution. The mean ± SD are reported for normally distributed continuous data. As described earlier in Chapter 3, the group differences were analysed as a preliminary step for the key variables and also for the retinal tissue thickness to examine the participant characteristics. However to examine the relationship between variables such as age and sex of the individuals with the retinal thickness, univariate general linear regression was performed. Full retinal, the RNFL and GCC thickness were entered as dependent variables in separate regression models. Age and sex were entered as independent variables.

4.1.5 Results

Eighteen (42%) of the study participants were males. The mean age in the control group was 55.2 ± 9.5 years; age group examined was 40-73 years. Forty individuals (95%) were Caucasians.

Full retinal thickness in females and males

Table 5 shows the retinal thickness in males and females in this control group. Figure 24 presents the mean and the 95% CI for retinal thickness at various regions in females and males.

Table 5. Full retinal thickness in females and males in the control group

Full retinal thickness parameters (μm)	Females n = 24 Mean ± SD Min - Max	Males n = 18 Mean ± SD Min - Max
Thickness within central 1 mm zone	251 ± 25 180 - 313	260 ± 27 194 - 296
Overall thickness at parafovea	313 ± 13 287 - 341	317 ± 16 277 - 336
Retinal thickness in superior parafovea	316 ± 13 285 - 339	321 ± 15 285 - 341
Retinal thickness in inferior parafovea	309 ± 13 288 - 342	313 ±17 269 - 336
Overall thickness at perifovea	273 ± 12 245 - 301	270 ± 13 236 - 293
Retinal thickness in superior perifovea	279 ± 12 251 - 306	277 ± 15 242 - 304
Retinal thickness in inferior perifovea	266 ± 15 239 - 312	263 ± 17 222 - 288

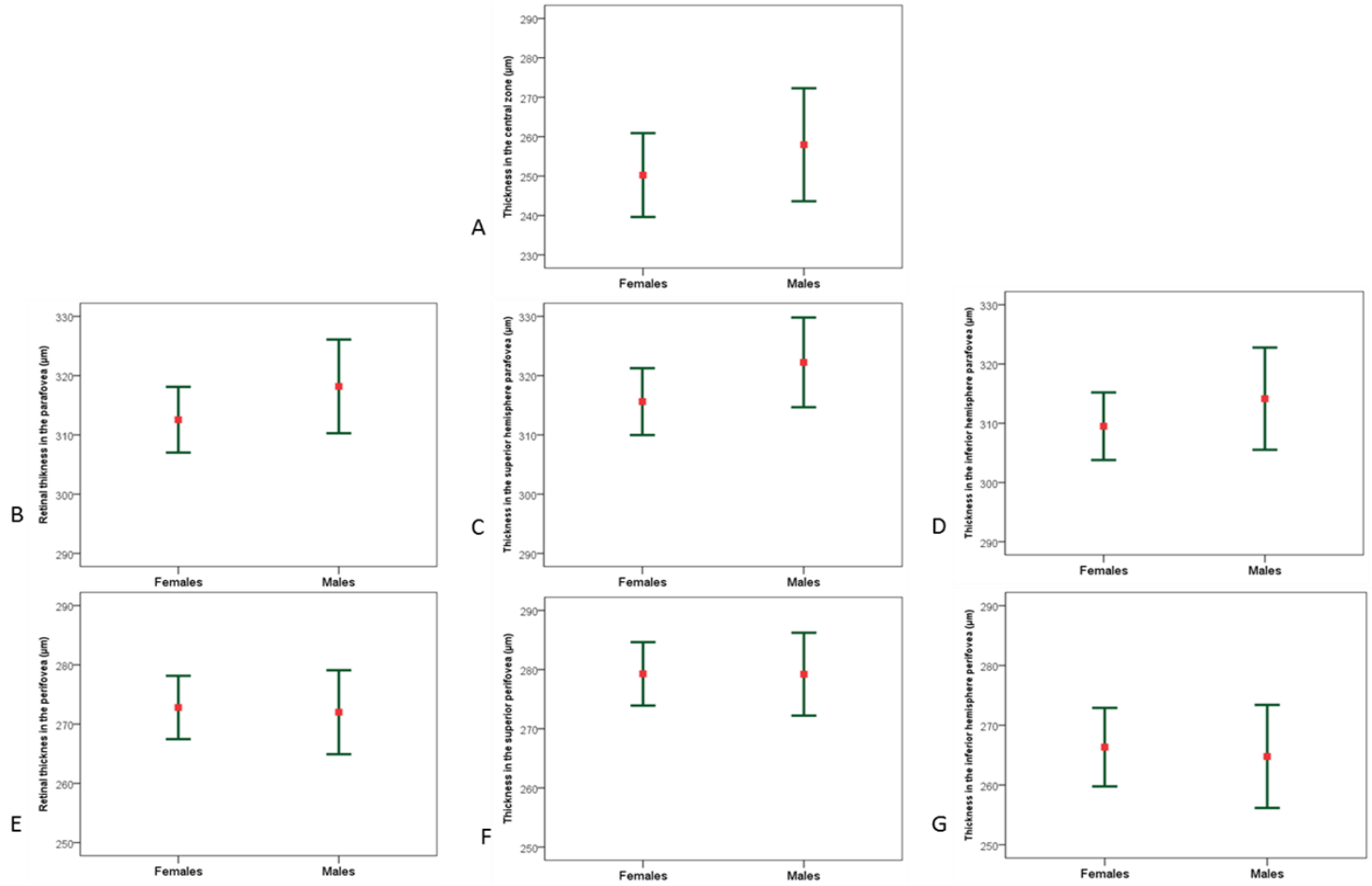


Figure 24. Full retinal thickness in females and males in the control group for (A) central 1mm zone (B) overall parafovea (C) superior hemisphere parafovea (D) inferior hemisphere parafovea (E) overall perifovea (F) superior hemisphere perifovea (G) inferior hemisphere perifovea. Marker indicates mean retinal thickness in µm. Error bars are 95% CI

Inner retinal thickness in males and females

Table 6 shows summary statistics for the inner retinal thickness in females and males. Figure 25 presents the mean and the 95% CI for RNFL and GCC thickness in females and males.

Table 6. Inner retinal thickness in females and males in the control group

Inner retinal thickness (μm) parameters	Females n = 24 Mean ± SD Min - Max	Males n = 18 Mean ± SD Min - Max
Overall RNFL thickness	104 ± 12 74 - 132	100 ± 9 80 - 119
Superior hemisphere RNFL	103 ± 15 66 - 135	101 ± 11 77 - 128
Inferior hemisphere RNFL	104 ± 12 82 - 136	99 ± 12 83 - 118
Overall GCC thickness	97 ± 6 83 - 108	95 ± 7 76 - 106
Superior hemisphere GCC	97 ± 7 78 - 110	94 ± 8 70 - 106
Inferior hemisphere GCC	97 ± 6 85 - 107	97 ± 7 81 - 108

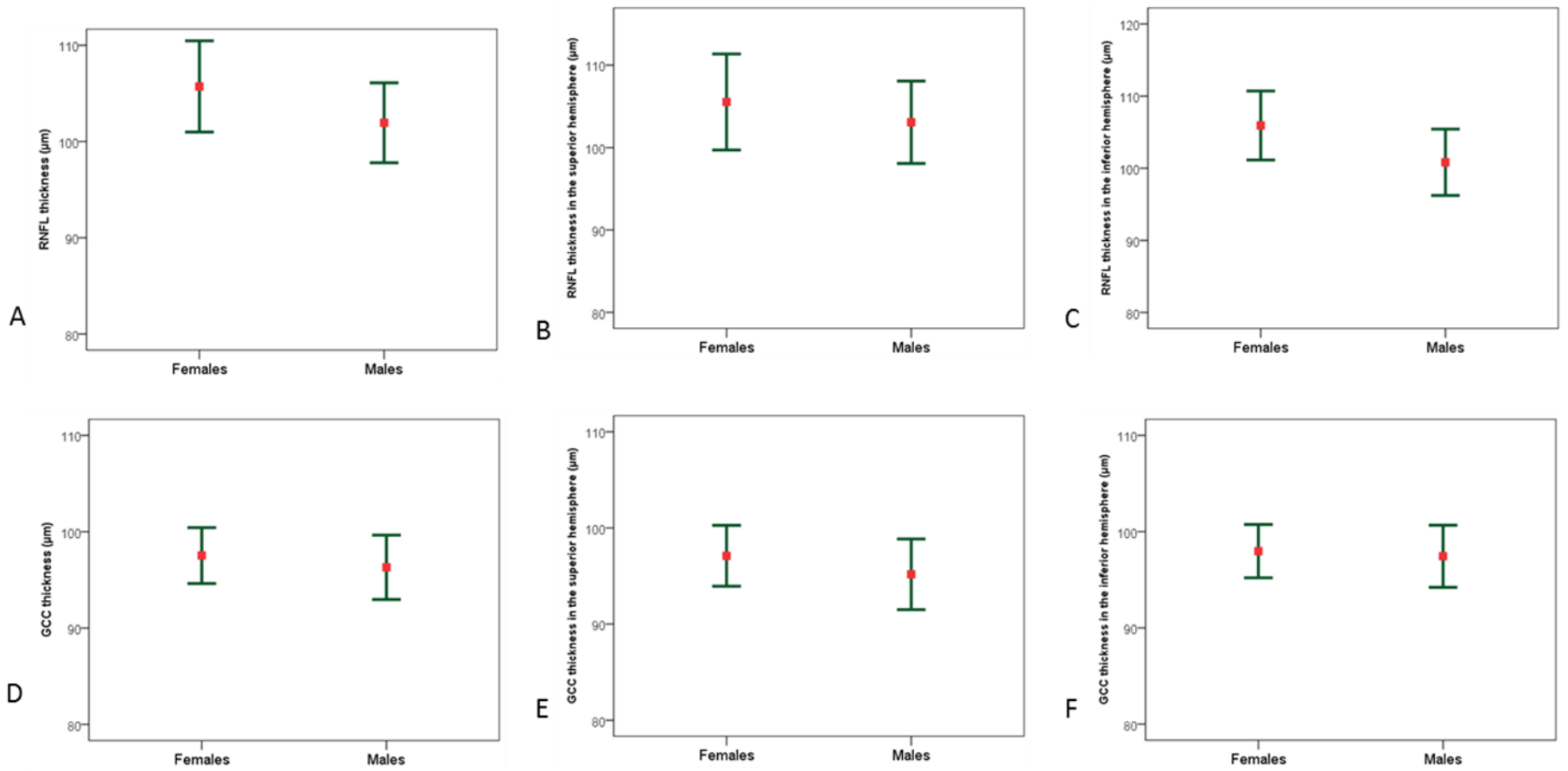


Figure 25. Inner retinal thickness in females and males in the control group for (A) overall RNFL (B) superior hemisphere RNFL (C) inferior hemisphere RNFL (D) overall GCC (E) superior hemisphere GCC (F) inferior hemisphere GCC. Marker indicates mean inner retinal thickness in µm. Error bars are 95% CI

Relationship between age, sex of the individuals and the retinal tissue thickness

The relationship between age, sex of the individuals for the full retinal tissue thickness and the inner retinal tissue thickness were analysed for each region in separate regression models. The full retinal thickness within the central 1mm zone was not significantly related to age or sex of the individuals. However, the overall and the hemisphere thickness in the parafovea and perifoveal regions significantly reduced with advancing age at the rate of 0.58-0.67 µm and by 0.65-0.78 µm respectively. Males had significantly thicker (8 µm) superior parafovea than females (p = 0.041) when adjusted for age. Other retinal regions did not show significant relation to sex of the individuals. Results for full retinal thicknesses are presented in Table 7.

Table 7. Full retinal thickness with age and sex as variables in the model

Full retinal thickness in control group (µm)	Variables entered in regression	B	SE(B)	p-value
Thickness within central 1 mm zone	Age	-0.476	0.400	0.240
	Males	11.143	7.830	0.162
Overall thickness at parafovea	Age	-0.588	0.204	0.006
	Males	6.935	3.941	0.086
Retinal thickness in superior parafovea	Age	-0.670	0.203	0.002
	Males	8.318	3.944	0.041
Retinal thickness in inferior parafovea	Age	-0.589	0.218	0.010
	Males	5.719	4.294	0.190
Overall thickness at perifovea	Age	-0.700	0.185	<0.001
	Males	0.178	3.715	0.962
Retinal thickness in superior perifovea	Age	-0.788	0.183	<0.001
	Males	0.593	3.677	0.873
Retinal thickness in inferior perifovea	Age	-0.651	0.237	0.009
	Males	1.080	3.677	0.822

SE, standard error; B, slope

The overall and hemisphere RNFL and GCC thicknesses reduced significantly with advancing age but did not show significant relationship to sex of the individuals. The overall RNFL thickness reduced at the rate of 0.61 μm per year increase in age (0.68 μm per year and 0.53 μm per year for the superior and inferior hemispheres respectively). The overall GCC thickness reduced at the rate of 0.49 μm per year increase in age (0.53 μm per year and 0.46 μm per year reduction in GCC thickness for the superior and inferior hemispheres respectively). The results for the RNFL and GCC thickness parameters are presented in Table 8.

Table 8. Inner retinal thickness with age and sex as variables in the model

Inner retinal thickness in control group (μm)	Variables entered in regression	B	SE(B)	*p*-value
Overall RNFL thickness	Age	-0.614	0.152	<0.001
	Males	-1.872	2.998	0.536
Superior hemisphere RNFL	Age	-0.689	0.189	0.001
	Males	-0.040	3.741	0.992
Inferior hemisphere RNFL	Age	-0.538	0.157	0.001
	Males	-3.702	3.062	0.233
Overall GCC thickness	Age	-0.496	0.085	<0.001
	Males	-0.169	1.702	0.921
Superior hemisphere GCC	Age	-0.532	0.098	<0.001
	Males	-1.289	1.964	0.515
Inferior hemisphere GCC	Age	-0.460	0.081	<0.001
	Males	0.936	1.61	0.564

SE, standard error; B, slope

4.1.6 Discussion

The study examined the relationship between age, sex of the individuals and the full retinal thickness and inner retinal thickness in a group of individuals without diabetes or neuropathy.

The central zone thickness and age

In a cohort of people without diabetes, belonging to the age group 40-73 years, retinal thickness within central 1mm was not significantly related to age or sex of the individuals. This is in agreement with past studies where the fovea did not show significant relationship with age (Wong et al., 2004, Chan et al., 2006). In contrast, Eriksson et al (2009) found that thickness within central 1mm zone reduced significantly at the rate of 0.26 μm per year increase in age. However, the participants belonged to age group 12 years to 74 years. This wider range of age group examined may be a possible explanation for the differences in effect due to age and therefore may explain differences in slope. Also, Eriksson and his group in their study, performed regression analysis for each ETDRS sector with age that were not adjusted for sex of the individuals. The current project used regression models that were adjusted for the effects of age and sex of the individuals.

Full retinal thickness and age

In the control group, thickness in the parafovea and perifovea reduced with advancing age. This is consistent with that reported by Fraser-Bell et al (2005). In the current study, thickness in the parafovea reduced at the rate of 0.58 μm per year (0.67 and 0.58 μm per year for the superior and inferior hemispheres respectively). The thickness in the overall perifovea reduced at the rate of 0.70 μm per year increase in age (0.78 μm and 0.65 μm in the superior and inferior perifovea respectively). This is comparable to that reported by Alamouti et al (2003) who observed that the overall retinal thickness reduced at the rate of 0.53 μm per year increase in age in those without diabetes. Eriksson et al (2009) observed that the retinal thickness reduced with age both in the parafovea (0.25 μm and 0.35 μm reduction per year increase in age in superior and inferior

parafovea respectively) and in the perifovea (0.29 μm and 0.46 μm reduction in the superior and inferior perifovea respectively). However, the observations were unadjusted for sex of the individuals, which may be an explanation for the differing results when compared to the current study.

Full retinal thickness in males and females

In the current study, retinal thickness in the superior parafovea was significantly thicker (8 μm) in males compared to females, when adjusted for the effect of age. The other retinal regions were not significantly related to sex of the individuals. Studies from the past (Massin et al., 2004, Wong et al., 2004, Bressler et al., 2008, Huang et al., 2009) observed thicker retina in males compared to females.

Wong et al (2004) studied 117 eyes in the age group 13-81 years and observed thickness within central 1mm zone to be greater (mean = 9 μm) for males than females. Therefore, in the current study, it is likely that a larger sample of individuals may have demonstrated significant differences between males and females in the central and the other retinal regions.

Inner retinal thickness and age

In this study, both the RNFL and GCC thicknesses reduced with increasing age but did not show significant relationship with sex of the individuals. The overall RNFL thickness reduced at the rate of 0.61 μm per year increase in age and the overall GCC thickness reduced at the rate of 0.49 μm per year increase in these individuals aged 40-73 years. Previous study reported that RNFL reduced at the rate of 0.2 μm per year in a group of people belonging to age group 18-85 years (Bundez et al., 2007). Another study observed that the GCC thickness reduced at a rate of 1.59 μm per decade belonging to the age group 22-84 years (Kim et al., 2011). It has been reported that retinal ganglionic axons are lost at the rate of 2000 axons per year before the age of 50 years and at the rate of 7000 axons per year after the age of 50 years and on an average, about 5000 axons per year are lost from birth to death (Quigley et al., 1989). This may be a likely explanation for the differences in the magnitude of the 'age effect' observed across studies.

In the study by Bundez and his group, nearly 65% of the participants were Caucasians. In the study by Kim and his group regarding the GCC parameters, all participants examined were of Korean descent. The RNFL thickness is reported to be thicker in Asians and Hispanics compared to Caucasians (Bundez et al., 2007). In the current study, 95% of individuals in the control group were Caucasians. Therefore, the results from the current project may not be applicable to other ethnic groups.

4.1.7 Conclusion

Full retinal thickness and inner retinal thicknesses decrease with increasing age, when adjusted for sex of the individuals, in this cohort of individuals without diabetes or neuropathy. The superior parafovea is thicker in males than in females. This control group predominantly comprised of Caucasians in the age group 40-73 years. Larger sample sizes may be required to demonstrate between-gender differences in thickness in the central zone and other retinal regions.

With this knowledge, the variables age and sex were therefore included in subsequent analyses in the diabetic group for exploring the relationship between retinal thickness and certain key clinical variables.

Examining the relationship between retinal tissue thickness and diabetic peripheral neuropathy in a group of individuals with diabetes was the main objective of this research program. Though data on the control group is also presented for comparison purposes, the following section 4.2 and onwards will specifically discuss the findings in individuals with diabetes.

4.2 Retinal tissue thickness: Type 1 versus type 2 diabetes

Rationale

The group with diabetes comprised of individuals belonging to either type 1 or type 2 diabetes. However, the two types of diabetes differ in their aetiology.

Type 1 diabetes is an autoimmune process causing destruction to pancreatic beta cells, ultimately leading to absolute insulin deficiency; therefore, insulin is necessary for survival. The autoimmune process can be identified by the presence of antibodies against islet cell, insulin, or anti-glutamic acid decarboxylase (anti-GAD) (Zimmet et al., 1994).

Type 2 diabetes is the most common form of diabetes. There is only a relative insulin deficiency in these individuals. Type 2 diabetes generally has no markers such as anti-GAD or insulin antibodies as are observed in type 1 diabetes. Individuals with this disorder either have insulin resistance or impaired secretion or both, which can be identified when the disease manifests. Exercise, weight reduction or oral hypoglycaemic agents may help to improve sensitivity of tissues to insulin (Joslin et al., 1936).

Mitchell et al (2008) proposed that people with type 1 diabetes may have responded better to metabolic control. Hence, type 1 diabetes may be viewed as a metabolic disorder; however, most people with diabetes who died of cardiovascular and other vascular complication belonged to type 2 diabetes. Therefore, type 2 diabetes may be viewed as a vascular disorder (Fox, 2010). This raises the question whether they can be combined to represent one group of individuals with diabetes.

One way to answer this research question is to explore the relationship between the type of diabetes and the retinal tissue thickness. Therefore, the first part of the experiment was modified to explore the effect of type of diabetes on the retinal tissue thickness. If the type of diabetes is significantly related to the retinal tissue thickness, then it would be rational to report results for type 1 and

type 2 diabetes separately. The following sections present a review of literature that report certain differences in type 1 and type 2 diabetes.

There have been a limited number of studies (described below in section 4.2.1) that reported certain differences in ocular structure and visual function in individuals with type 1 diabetes compared to type 2 diabetes. The following section provides a review of those findings reported in the literature.

4.2.1 Review of literature

Clinical and demographic variables: type 1 versus type 2 diabetes

In a recent study by Bronson-Castain et al (2012) that examined retinal function and structure using mfERG, retinal vessel diameter measurement and OCT in adolescent type 1 and type 2 diabetic people without DR, the authors observed that variables such as duration of diabetes (mean duration, 5.7 ± 3.6 years versus 2.1 ± 1.3 years) and HbA_{1c} levels (9.6 ± 2.2% versus 7.6 ± 3.0%) were significantly higher in adolescents with type 1 than those with type 2 diabetes.

On the other hand, those with type 2 diabetes had greater body mass index (BMI) compared to type 1 diabetes and those without diabetes (mean, 34.6 kg/m^2 versus 22.4 kg/m^2 and 22.6 kg/m^2 respectively), suggesting differences in clinical variables between the two groups.

Neuropathy and the type of diabetes

Prevalence of diabetic neuropathy

A difference in the prevalence of neuropathy between the two types of diabetes has been observed. A study from the past observed prevalence of neuropathy in type2 diabetes (50%) to be twice as high as in type 1 diabetes (25%) (Van Acker et al., 2009). However, it is to be noted that the number of people with type 2 diabetes examined was more than twice the number with type 1 diabetes (n = 767 versus 344 respectively).

Sural nerve changes in diabetic neuropathy

In the presence of hyperglycaemia, a significantly higher nerve fibre loss has been observed in the sural nerves in type 1 experimental diabetic rat models but not in type 2 models. The authors mention this as being related to axonal degeneration at the nodes caused by disruption of Na^+/K^+ - ATPase. This axonal change has been found to be correlated with subnormal conduction velocities in the sural nerves. This neuropathy-related structural change has been observed typically in type 1 rats but not in type 2 rats (Kamiya et al., 2005; Sima et al., 2006).

Ocular complications in type 1 and type 2 diabetes

Anterior segment complications

Corneal sensitivity was evaluated in a group of people with diabetes with proliferative retinopathy. Corneal sensitivity was significantly poorer in individuals with type 2 diabetes compared to those with type 1 diabetes and those without diabetes (Ruben, 1994). However, the results may have been confounded by the effect of patient age, in that individuals with type 2 diabetes were older than those in the other two groups (mean± SD, 58 ± 12 years versus 46 ± 15 years; median age, 57 years versus 51 years).

In individuals with type 1 diabetes, cataract with vacuoles and snow-flake like opacities with light scattering properties are seen. However, in those with type 2 diabetes, the cataractous changes are similar in appearance to that of age-related cataract (Bron et al., 1998). Both the groups were matched with respect to duration of diabetes.

Retinal complications

Prevalence of diabetic retinopathy

Independent studies reported different prevalence rates for DR in the two types of diabetes. The data combined from two studies conducted in the US, the

WESDR and New Jersey 725 study, observed prevalence of DR to be 82% among people with type 1 diabetes (Roy et al., 2004).

Analysis from pooled data from eight population-based studies in the US reported prevalence of DR to be 40% in people with type 2 diabetes above 40 years of age (Kempen et al., 2004); two studies conducted in Indian subcontinent reported prevalence as 22% (Narendran et al., 2002) and 27% (Dandona et al., 1999) in people with type 2 diabetes above 50 years of age. Also, the visual impairment from DR in type 1 diabetes is most likely from proliferative DR, whereas in type 2 diabetes, it is more likely to be from macular oedema (Cunha-Vaz et al., 2012).

Retinal tissue structure and function

Metabolic control can influence both retinal structure and function. With 2 years of intensive metabolic control, the contrast sensitivity function in individuals with type 1 diabetes improved in those without DR and in mild DR when compared to baseline values (Verrotti et al., 1998). In a study by Sugimoto et al (2010) in people with type 2 diabetes, there was a significant reduction in superior retinal nerve fibre layer thickness after 4 months of strict metabolic control. The above two studies show retinal function and structural changes in response to strict metabolic control in individuals with type 1 diabetes and type 2 diabetes respectively. This might possibly indicate differences in the way the two types of diabetes respond to strict metabolic control.

Bronson-Castain et al (2012) analysed the frequency of abnormality (defined as equal to as or more than ± 2 z-score distributions) in the mfERG implicit times, mfERG amplitudes (which provide a measure of retinal function) and retinal thicknesses among adolescents with type 1 diabetes, type 2 diabetes and in participants without diabetes (control). The authors observed frequency of abnormality was greater in those with type 2 diabetes than those with type 1 diabetes and control group, for all three parameters (Bronson-Castain et al., 2009).

Longer duration of diabetes is more commonly observed in individuals with type 1 form than the type 2 form of diabetes. RNFL thickness has been reported to be inversely related to the duration of diabetes (Oshitari et al., 2009). If the nerve fibre layer thickness parameters are linked with the duration of diabetes, it is likely that the neural tissue thickness in people with type 1 diabetes may be reduced compared to that of people with type 2 diabetes.

To summarize, there appears to be certain differences between type 1 and type 2 diabetes. Given that the retinal tissue integrity has not been studied in detail in the two types of diabetes, examination of the full retinal and the inner retinal thicknesses in the two types of diabetes will provide a better understanding for further experiment. Therefore, the current research program sought to explore the association between the type of diabetes and retinal tissue thickness.

4.2.2 Purpose

The purpose of this part of the study was to examine the relationship between the type of diabetes and retinal tissue thickness

4.2.3 Research question and hypothesis

Is there a relationship between the type of diabetes and the retinal tissue thickness?

It was hypothesised that the type of diabetes is significantly related to the retinal tissue thickness.

4.2.4 Methods

Information regarding the type of diabetes was self-reported or obtained from health care practitioner reports. After stratifying based on the type of diabetes, 84 individuals were designated as having type 1 diabetes and 67 individuals had type 2 diabetes. There were 42 individuals in the control group. For the purposes of this experiment, individuals with type 1 diabetes are referred to as ‘T1DM’ and those with type 2 diabetes as ‘T2DM’.

As a preliminary step, the key clinical and demographic variables of interest namely age, sex, NDS score, DR, duration of diabetes and HbA_{1c} levels were analysed and compared between the three groups. The data for various other clinical and demographic variables were also compared between groups.

The full retinal and inner retinal tissue thicknesses were compared between T1DM and T2DM and also with the control group. The relation between the type of diabetes and the retinal tissue thickness, adjusted for age, sex of the individuals, DR, NDS, duration of diabetes and HbA_{1c} levels was subsequently explored.

4.2.5 Statistical analysis

The proportion of individuals with DR in the two groups of diabetes was analysed. A chi-square test of independence was performed to analyse the relation between DR and the type of diabetes. An analysis of variance test was used to compare continuous and normally distributed data between type 1 diabetes, type 2 diabetes and the group without neuropathy or diabetes (named as the 'control group'); a Tukey's HSD test was used after a significant result in the analysis of variance test. These data are represented as mean, standard deviation (SD) and range. For the reason that there is a possibility of type I error due to multiple comparisons, a Bonferroni post-hoc test has also been performed and the significance values are shown in the respective tables, to aid in the additional interpretation of the results.

For variables that did not follow a normal distribution, the group-differences were analysed using a Kruskal-Wallis test; a Mann-Whitney U test was performed after a significant Kruskal-Wallis test. These data are represented as median and inter-quartile range.

To examine the relationship between the type of diabetes and retinal tissue thickness, a univariate general linear model was utilized. The overall retinal thickness, the RNFL and GCC thicknesses were entered as dependent variables and analysed in separate regression models. The type of diabetes, presence of diabetic retinopathy and sex of the individuals were coded and entered as

factors; age, NDS (entered as continuous variable), duration of diabetes and HbA_{1c} levels were entered as covariates in each of the regression model.

Main effects were assessed for type of diabetes, age, DR status, gender, NDS, duration of DM and HbA_{1c} levels on the thicknesses of the overall fovea, parafovea, perifovea, and the RNFL and GCC thicknesses.

Interactions were analysed between

a) type of diabetes and age
b) type of diabetes and NDS
c) type of diabetes and duration of diabetes
d) type of diabetes and DR

4.2.6 Results

Clinical and demographic variables

Results are presented in Table 9. The magnitude of differences will be reported only for significantly different variables.

People with T2DM were significantly older than those with T1DM and the control group (mean difference of about 6 years and 4.5 years respectively). The mean HbA_{1c} levels were not significantly different, but, when compared to control group, both the groups with diabetes had significantly elevated HbA_{1c} levels.

Forty six percent of people with T1DM had DR compared to 35% among those with T2DM. A chi-square test of independence was performed to analyse the relationship between the type of diabetes and the proportion of people with retinopathy. There was no statistically significant relationship between the two, $\chi^2_{(1, 151)} = 1.725$, $p = 0.189$. There were 53% of males and 64% of males in T1DM and T2DM groups respectively and the relationship was not significant, $p = 0.189$.

Table 9. A summary of key clinical and demographic characteristics in groups with type 1 and type 2 diabetes and in control group

Clinical variables	T1DM (A) Mean ± SD n Min - Max	T2DM (B) Mean ± SD n Min - Max	Controls(C) Mean ± SD n Min - Max	ANOVA *F*	ANOVA *p*-values	Bonferroni *p*-values
Age	53.6 ± 9.2 84 40.1 - 77.3	60.4 ± 8.1 67 41.4 - 72.1	55.9 ± 9.6 42 40.7-72.6	10.739	<0.001*	A vs. B, <0.001 B vs. C, 0.034
Males n (%)	45 (53%)	43 (64%)	18 (42%)		0.189 ‖	
HbA_{1c} (%)	8.0 ± 1.2 84 6 - 13	7.5 ± 1.4 66 5 - 12	5.4 ± 0.3 42 5 - 6	67.381	<0.001 §	A vs. C,<0.001 B vs. C,<0.001
DR (%)	39 (46%)	24 (35%)	n/a		0.189 ‖	

Controls - no diabetes/neuropathy
* Significant differences for A vs. B and B vs. C with Tukey's HSD
§ Significant differences between A,B vs. C with Tukey's HSD
‖ Significant differences with χ^2 test for A vs. B
n/a not applicable; DR, diabetic retinopathy (representing those with clinically visible signs of retinopathy)
Proportion of males in this table compared between T1DM and T2DM

Table 10 shows duration of diabetes and NDS in the three groups using non-parametric tests. Individuals with T1DM had significantly longer duration of diabetes (mean difference of 5 years) than T2DM group. The NDS scores were a unit higher NDS in the T2DM group compared to the T1DM group and was 2 units greater when compared to that of the control group.

Table 10. Duration of diabetes and NDS comparison with non-parametric tests

Clinical variables	T1DM (A) Median IQR Min-Max	T2DM (B) Median IQR Min-Max	Control(C) Median IQR Min-Max	Kruskal-Wallis test		
				χ^2	*df*	*p*-values
Duration of diabetes (years)	17 26 0 - 56	12 12 2 - 64	n/a	<0.027 ‡		
NDS	1 2 0 - 10	2 5 0 - 10	0 1 0 - 2	28.55	2	<0.001 §‡

T1DM, type 1 diabetes; T2DM, type 2 diabetes; Controls - no diabetes/neuropathy; NDS, neuropathy disability score, IQR, inter-quartile range
‡ Significant differences between A and B, with Mann-Whitney U test
§ Significant differences between A, B vs. C with Mann-Whitney U test

The summary statistics for the other clinical and neuropathy related measures in the type 1, type 2 and control groups by non-parametric tests are presented in Table 11. The body mass index (BMI) was 4 kg/m^2 higher in T2DM compared to T1DM and 5 kg/m^2 higher than the control group. Among the neuropathy measures, those with T2DM had a unit higher DNSS than those with T1DM. The QST vibration threshold was 3.8 Hz higher in T2DM compared to the control group and 3 Hz higher in T1DM group compared to the control group. Both the groups with diabetes had lower number of monofilament points detected than the control group. The QST warm sensation threshold, warm and cold-induced pain thresholds were similar in the three groups.

Table 11. Summary of other clinical and demographic characteristics in type 1, 2 diabetes and in control group using non-parametric tests

Clinical variables	T1DM (A) Median IQR Min-Max	T2DM (B) Median IQR Min-Max	Controls(C) Median IQR Min-Max	Kruskal-Wallis test		
				χ^2	*df*	*p*-values
Body mass index (kg/m^2)	27.8 5.4 19 - 47	31.0 6.8 21 - 55	26.9 4.3 19 - 46	28.16	2	<0.001 §‡
DNSS	0 1 0 - 3	1 2 0 - 4	0 0 0 - 1	29.67	2	<0.001 §‡
QST warm sensation (˚C)	39.7 6.7 33.1 - 50.0	39.2 7.8 19.4 - 50.0	37.2 6.7 30.5 - 47.0	18.56	2	0.076
QST cold induced pain threshold (˚C)	8.1 17.8 0.0 - 26.5	4.7 19.7 0.0 - 29.3	7.9 17.9 0.0 - 29.4	0.05	2	0.975
QST warm induced pain threshold (˚C)	48.9 3.3 39.5 - 50.0	49.5 3.0 37.3 - 50.0	48.7 4.0 41.2 - 50.0	1.60	2	0.448
QST vibration threshold (Hz)	9.0 16.4 1.0 - 130.0	9.8 19.1 1.4 - 130.0	6.0 7.6 1.2 - 42.2	12.84	2	0.002 §
Monofilament (no. of points detected out of 3)	3 0 0 - 3	3 0 0 - 3	3 0 2 - 3	9.36	2	0.009 §

T1DM, type 1 diabetes; T2DM, type 2 diabetes; Controls - no diabetes/neuropathy; IQR, inter-quartile range; DNSS, diabetic neuropathy symptom score; QST, quantitative sensory testing
‡ Significant differences for T1DM versus T2DM by Mann-Whitney U test
§ Significant differences between both T1DM, T2DM groups versus control group by Mann-Whitney U test

Table 12 presents a summary of other clinical and neuropathy measures. Individuals with T2DM had 0.8 mmol/L lower total cholesterol levels than the T1DM as about 63% of individuals in T2DM group and 49% in T1DM group were on lipid-lowering drugs compared to 7% in the control group. Both the groups with diabetes had significantly lower total cholesterol levels when compared to the control group (1.7 and 0.9 mmol/L respectively). Systolic and diastolic blood pressure was not significantly different in the three-group comparison. Among the neuropathy measures, T1DM and T2DM diabetic groups had lower peroneal nerve conduction velocities (3.9 m/s and 3.5 m/s respectively) and 3.5 ˚C lower QST cold thresholds compared to control group. The peroneal nerve amplitudes did not differ significantly between groups.

Table 12. Other clinical and neuropathy measures in groups with type 1 and type2 diabetes and the control group

Clinical variables and neuropathy measures	T1DM (A) Mean ± SD n Min - Max	T2DM (B) Mean ± SD n Min - Max	Controls (C) Mean ± SD n Min - Max	ANOVA *F*	ANOVA *p*-values	Bonferroni *p*-values
Total cholesterol (mmol/L)	4.7 ± 1.0 84 3.0 - 8.6	3.9 ± 0.8 66 2.6 - 6.2	5.6 ± 1.0 42 4.2 - 9.2	43.067	<0.001 ‡§	A vs. C,<0.001 B vs. C,<0.001
BP resting systolic (mmHg)	128 ± 16 84 95 - 167	128 ± 14 67 87 - 161	126 ± 14 42 102 - 163	0.448	0.640	
BP resting diastolic (mmHg)	78 ± 8 84 53 - 97	75 ± 8 67 52 - 91	79 ± 9 42 63 - 108	3.064	0.051	
Peroneal M amp ankle to EDB (millivolts)[a]	3.6 ± 2.4 83 0.1 - 10.4	4.0 ± 2.6 67 0.1 - 14.1	4.2 ± 2.5 42 0.1 - 9.2	0.804	0.449	
Peroneal CV ankle to FH (meters/second)	43.6 ± 5.6 84 20.0 - 52.3	44.0 ± 6.5 67 20.0 - 57.1	47.5 ± 7.4 42 20.0 - 59.0	5.873	0.003 §	A vs. C, 0.004 B vs. C, 0.015
QST cold sensation threshold average (˚C)	24.7 ± 7.4 84 0.0 - 31.3	24.8 ± 7.4 67 0.0 - 31.3	28.2 ± 2.7 42 19.6 - 33.2	4.556	0.012 §	A vs. C, <0.016 B vs. C, <0.030

T1DM, type 1 diabetes; T2DM, type 2 diabetes; BP, Blood pressure; EDB, extensor digitorum brevis; FH, fossa head; Controls - no diabetes/neuropathy;
§ Significant differences between T1DM and T2DM versus control group with Tukey's HSD test
‡ Significant difference between T1DM versus T2DM with Tukey's HSD test
Where there was no response, the following substituted data were used: Peroneal amp:0.1 mV; Peroneal CV: 20 m/s reflecting lowest recorded value in our laboratory

The retinal tissue thicknesses analysis showed people with T2DM had reduced thickness in the overall parafovea, perifovea, the RNFL and GCC thickness when compared to individuals with T1DM. The mean differences were 6 μm, 7 μm, 6 μm and 4 μm for the parafovea, perifovea, RNFL and GCC thicknesses respectively. Thickness within the central zone and parafovea in T2DM was further reduced when compared to the control group. Table 13 provides a summary of the full retinal thickness and in the three groups.

Table 13. Retinal tissue thickness in groups with type 1 and type 2 diabetes and the control group

Retinal variables (μm)	T1DM (A) Mean ± SD n Min - Max	T2DM (B) Mean ± SD n Min - Max	Control (C) Mean ± SD n Min - Max	ANOVA *F*	ANOVA *p*-values	Bonferroni *p*-values
Thickness within central 1mm	249 ± 23 84 140 - 293	241 ± 24 66 177 - 289	254 ± 26 42 180 - 313	4.064	0.019 †	B vs. C, 0.018
Overall parafovea	311 ± 16 84 259 - 338	305 ± 16 66 268 - 342	314 ± 14 42 277 - 341	5.291	0.006 †‡	B vs. C, 0.007
Overall perifovea	272 ± 13 84 230 - 301	265 ± 15 66 201 - 297	272 ± 13 42 236 - 301	5.081	0.007 ‡	A vs. B, 0.008
Overall RNFL thickness	105 ± 10 84 82 - 132	99 ± 12 67 71 - 136	102 ± 11 42 74 - 132	3.716	0.026 ‡	A vs. B, 0.021
Overall GCC thickness	97 ± 8 84 77 - 117	93 ± 8 66 71 - 113	96 ± 7 42 76 - 108	4.281	0.015 ‡	A vs. B, 0.013

T1DM, type 1 diabetes; T2DM, type 2 diabetes; RNFL, retinal nerve fibre layer; GCC, ganglion cell complex
† Significant differences between B and C by Tukey's HSD
‡ Significant differences between A and B by Tukey's HSD
Controls - no diabetes/neuropathy

For a better understanding of the relative effects of these variables, regression models were utilized.

Effect of the type of diabetes

Regression was performed in the group with diabetes; NDS was included as a continuous variable from 0-10.

It was observed that neither the interactions nor the main effect of the type of diabetes was significantly related to any of the retinal parameters. Although the main effect of the type of diabetes at the parafovea was p = 0.056, the relationship did not reach statistical significance. Therefore, the regression models were terminated at this stage. Table 14 presents the significance values of the main effects and the interactions between the type of diabetes and a range of variables namely, age, DR, duration of diabetes and NDS.

Table 14. Significance values for the main effects of type of diabetes and interactions with a range of factors in the model

Interactions between variables	*p*-values for interactions and main effects at various retinal regions				
	Thickness within the central 1mm zone	Thickness in the parafovea	Thickness in the perifovea	RNFL thickness	GCC thickness
Type of DM and age	0.655	0.180	0.467	0.314	0.511
Type of DM and NDS	0.510	0.884	0.473	0.608	0.908
Type of DM and duration of DM	0.330	0.909	0.632	0.905	0.547
Type of DM and DR	0.222	0.397	0.290	0.635	0.841
Main effect of the type of DM	0.160	0.056	0.085	0.688	0.165

DM, diabetes mellitus; NDS, neuropathy disability score; DR, diabetic retinopathy

4.2.7 Discussion

The study sought to explore the relationship between the type of diabetes and retinal tissue thickness parameters while adjusting for age, sex, DR, NDS, duration of diabetes and HbA_{1c} levels. It was observed that individuals with T2DM showed a tendency for lower retinal tissue thickness than those with T1DM at the parafovea; however, this did not reach statistical significance, after adjusting for the aforementioned factors. Neither the main effect nor the interactions of type of diabetes with other variables were significantly related to retinal thickness parameters. To date, this is the first study to investigate the relationship between retinal tissue thicknesses and the type of diabetes.

In the current study, analysis of clinical parameters showed that people with T2DM were older than T1DM. However, people with T1DM had a more prolonged duration of diabetes; this is sensible considering the nature of the disease and is also consistent with that reported in a past study (Van Acker et al., 2009). Although T1DM had a slightly greater proportion of people with DR than those with T2DM, the difference was not statistically significant. In the current study, there were differences in certain other clinical parameters and neuropathy measures between T1DM and T2DM groups. For instance, T2DM had higher NDS, DNS scores and elevated QST vibration thresholds compared to T1DM. The study by Van Acker et al (2009) observed the prevalence of neuropathy in T2DM to be twice as that observed in people with T1DM. These reported and observed differences may demonstrate certain dissimilarity in neuropathy measures between the two types of diabetes.

The unadjusted thickness in the parafovea, perifovea, RNFL and GCC were lower in T2DM than T1DM, with a mean difference of 7 μm for the parafovea, perifovea, 6 μm for the RNFL and 4 μm for GCC thickness. This finding is broadly consistent with that reported by Bronson-Castain et al (2013). The authors observed retinal thickness to be reduced in T2DM in comparison to T1DM; however, the mean difference was 3 μm.

In the current study, T2DM were slightly older than T1DM as well as the control group; therefore there is a possibility of confounding due to differences in age. It is debatable that any reduction in retinal thickness in T2DM may be attributed to difference in age. In the study by Bronson-Castain (2009) that compared retinal thickness in T1DM, T2DM and control groups, retinal thickness in individuals with T2DM was lower than those with T1DM. However, it is interesting to note that all participants were adolescents (mean age = 16years) and the groups were age-matched. Therefore, any observation in T2DM cannot be argued as being related to older age. The results likely represent true differences due to the type of diabetes. The results from their study suggest evidence of structural neural degeneration and functional deficits in the retina of people with T2DM compared to people with T1DM. The current study findings are broadly in agreement with their study. Although the differences in the current study are small but statistically significant, the clinical relevance of this difference is still debatable. Nevertheless, results from the literature and from the current study demonstrate certain differences in the structural aspects of retinal layers between T2DM and T1DM.

A likely explanation for this relatively greater compromise in type 2 diabetic people could be that there may be an initial phase of reversible functional compromise due to greater neuro-plasticity and the possibility of reversal of changes with good metabolic control, especially in T1DM compared with T2DM. Following this, structural changes may become more permanent; this may no longer be reversible with metabolic control (Sima et al., 2006). Although the theory hypothesized by Sima et al was in relation to diabetic neuropathy in rats, the theory may hold true for ocular neural changes in humans. In addition, T2DM may remain unrecognized for several years (Goldstein et al., 2013). Therefore, it is likely that the retinal degeneration has already occurred and is possibly beyond any repair.

Therefore, a broad understanding is that there are certain pathophysiological differences between the two types of diabetes but the type of diabetes did not show significant relationship to retinal thickness parameters when adjusted for certain key variables.

4.2.8 Conclusion

People with T1DM and T2DM differ in certain clinical, demographic and neuropathy characteristics; however, the type of diabetes is not significantly related to retinal tissue thicknesses when adjusted for known confounding factors in this cohort of people with diabetes aged 40-77 years. Therefore, for exploring further research questions, the two groups of diabetes were combined to represent one group of people with diabetes.

4.3 Retinal tissue thickness and diabetic peripheral neuropathy

The primary objective of this research program was to explore the relationship between retinal tissue thickness and diabetic peripheral neuropathy. The following section 4.3 discusses the analysis on the full retinal thickness and the inner retinal thickness in relation to diabetic neuropathy defined using NDS criteria.

4.3.1 Purpose

The purpose of this study was to explore the relationship between retinal tissue thickness and diabetic peripheral neuropathy defined using NDS criteria.

4.3.2 Research question and hypothesis

Are the full retinal thickness and inner retinal thickness significantly related to the severity of diabetic neuropathy, defined using NDS criteria?

It was hypothesized that the full retinal thickness and the inner retinal thickness are significantly related to the severity of diabetic neuropathy defined using NDS criteria.

4.3.3 Methods

Full retinal thickness and the inner retinal thicknesses were measured with the OCT. When stratifying according to NDS criteria of neuropathy, 44 individuals were designated as having diabetic neuropathy, 107 individuals had diabetes but no neuropathy. There were 42 individuals in the control group.

The key variables namely age, sex of the individuals, NDS, DR, duration of diabetes and HbA_{1c} levels were analysed and compared as were the data for various other clinical and demographic variables.

The full retinal and inner retinal tissue thicknesses were then compared between the groups with and without neuropathy and with that of the control group. Subsequently, the relationship between severity of diabetic peripheral

neuropathy and retinal tissue thickness was explored in the group of individuals with diabetes, adjusting for age, sex, DR, duration of diabetes and HbA_{1c} levels.

4.3.4 Statistical analysis

The proportion of individuals with DR, proportion of males and females and ethnic composition were analysed using chi-square test of independence. The normality of the distribution was tested. An analysis of variance test was utilized to compare normally distributed data; a Tukey's HSD test was used after a significant result in the analysis of variance test. Data are represented as mean, standard deviation (SD) and range. There is a possibility of type I error due to multiple comparisons; therefore, a Bonferroni post-hoc test has also been performed and the significance values are presented in the respective tables to aid in the additional interpretation of results. For variables that did not follow a normal distribution, the group-differences were analysed using a Kruskal-Wallis test; a Mann-Whitney U test was performed after a significant Kruskal-Wallis test. These data are represented as median and inter-quartile range.

The data for the key variables namely age, sex, duration of diabetes, HbA_{1c} levels, DR, NDS, are discussed in detail; the results for the other clinical and neuropathy measures are presented in tables but are not discussed in detail.

The relationship between severity of diabetic peripheral neuropathy and the retinal tissue thickness was explored in the group of individuals with diabetes, using a univariate general linear model, adjusting for age, sex, DR, duration of diabetes and HbA_{1c} levels; the full retinal thickness and inner retinal thicknesses parameters were entered as the dependent variables in separate regression models. The presence or the absence of DR and sex of the individuals were coded and entered as factors in the model; age, duration of diabetes, NDS and HbA_{1c} levels were entered as covariates. The models were then modified to achieve the most parsimonious model.

4.3.5 Results

4.3.5.1 Ethnicity

The ethnic composition is presented in Table 15. Majority of the participants (86%-95%) in the three groups were Caucasians. To assess the relationship between ethnicity and neuropathy in the group with diabetes, the individuals who were not Caucasians were combined into one group and were compared to the group comprising of Caucasians. A chi-square test of independence showed that the relationship was not significant, $\chi^2_{(1, 151)} = 6.043$, $p = 0.418$.

Table 15. Ethnic composition in the groups stratified per NDS criteria and in control group

	Neuropathy per NDS n (%)	No neuropathy per NDS n (%)	Controls n (%)
Asian (e.g. Indian, Pakistani, Bangladeshi, Sri Lankan)	1 (2.3%)	2 (1.9%)	0%
Australian Aboriginal or Torres Strait Islander	0%	1 (1%)	0%
European (e.g. European Australian, English, German, Spanish)	39 (88.6%)	92 (86.0%)	40 (95.2%)
Middle Eastern (e.g. Iranian, Iraqi, Lebanese, Syrian)	0%	2 (1.9%)	0%
South East Asian (e.g. Chinese, Japanese, Korean, Indonesian, Thai, Malaysian)	0%	5 (4.6%)	1 (2.4%)
Other	3 (6.8%)	5 (4.6%)	0%
Not known	1 (2.3%)	0%	1 (2.4%)

Controls - no diabetes/neuropathy

4.3.5.2 Summary of clinical, demographic and neuropathy measures in the groups stratified per NDS criteria and in control group

Key variables

Tables 16 and 17 provide a summary of the key variables. The magnitude of the differences is discussed only for those that are significantly different and their *p*-values are presented in Tables. People with neuropathy were significantly older than those without neuropathy and the control group (mean difference of 4.5 years and 4 years respectively); also the individuals with neuropathy had 6 years prolonged duration of diabetes than those without neuropathy. The proportion of males was similar in the groups with diabetes with and without neuropathy, $\chi^2_{(1, 151)} = 2.505$, $p = 0.114$. The HbA_{1c} levels were not significantly different in the neuropathy group compared to no neuropathy group, but both the groups with diabetes had significantly higher levels compared to control group (mean difference of 2.6% and 2.3% respectively).

The NDS was 4.5 units greater in the neuropathy group than the group without neuropathy. The proportion of people with DR was higher among individuals with neuropathy (61%) than those without neuropathy (34%). A chi-square test of independence was performed to determine the relationship between diabetic retinopathy and neuropathy. The relationship was significant, $\chi^2_{(1, 151)} = 9.852$, $p = 0.002$.

Table 16. Summary of key variables in the groups stratified per NDS criteria and in control group

Variables	Neuropathy per NDS (A) Mean ± SD n Min - Max	No neuropathy per NDS (B) Mean ± SD n Min - Max	Controls(C) Mean ± SD n Min - Max	ANOVA *F*	ANOVA *p*-values	Bonferroni *p*-value
Age	59.9 ± 8.4 44 41.0 - 71.6	55.2 ± 9.4 107 40.1 - 77.3	55.9 ± 9.6 42 40.7 - 72.6	3.94	0.021 ‡	A vs. B, 0.018
Males, n (%)	30 (68%)	58 (54%)	20 (44%)		0.114 ‖	
HbA_{1c} (%)	8.0 ± 1.3 44 6 - 13	7.7 ± 1.3 106 5 - 12	5.4 ± 0.3 42 5 - 6	64.73	<0.001 §	A vs. C, B vs. C, <0.001
DR, n (%)	27 (61%)	36 (34%)			0.002 ‖	

DR, diabetic retinopathy (representing those with clinically visible signs of retinopathy)
‡ Significant differences between neuropathy and no neuropathy groups with Tukey's HSD
§ Significant differences between diabetic and control group with Tukey's HSD
‖ Significant with chi-square test
Proportion of males in this table compared between the groups with and without neuropathy;
Controls - no diabetes/neuropathy

Table 17. Duration of diabetes and NDS in the groups stratified per NDS criteria and in control group

Clinical and neuropathy variables	Neuropathy per NDS Median IQR Min - Max	No Neuropathy per NDS Median IQR Min - Max	Controls Median IQR Min - Max	Kruskal-Wallis		
				χ^2	df	*p*-values
Duration of diabetes (years)	19 20 6 - 64	13 18 1 - 53	n/a	<0.001 ‖		
NDS	4.5 4 3 - 10	0 1 0 - 2	0 1 0 - 2	116.32	2	<0.001 *

NDS, neuropathy disability score
‖ Significant differences with Mann-Whitney U test
* Significant differences between Neuropathy versus the other two groups with Mann-Whitney U test
Controls - no diabetes/neuropathy

Other clinical and neuropathy variables

Results are presented in Tables 18 and 19. Differences were observed in other clinical variables and neuropathy measures. For instance, the systolic BP was 7 mmHg higher in people with neuropathy compared to those without neuropathy and control group. Diastolic BP was similar in the three groups. The BMI in the neuropathy group was 3 kg/m^2 higher when compared to no neuropathy group and about 4 kg/m^2 higher when compared to control group. The group without neuropathy had 1.3 kg/m^2 higher BMI than the control group. The total cholesterol in neuropathy group was similar to no neuropathy group but was 1.3 mmol/L less in both groups with diabetes compared to the control group, as 52% of individuals in the neuropathy group, 61% in the no neuropathy group were on lipid-lowering drugs as compared to only 7% in the control group.

Individuals with neuropathy had a unit higher DNSS; the QST warm sensation threshold was 4.8 ˚C higher compared to no neuropathy group and was 5.9 ˚C higher than the control group. QST warm-induced pain thresholds were 1.5 ˚C higher compared to no neuropathy group and about 1.3 ˚C higher compared to control group; QST cold induced pain was similar in the three groups. QST cold sensation threshold was reduced by about 4.4 ˚C in the neuropathy group compared to no neuropathy group, and was reduced by 6.6 ˚C compared to the control group. QST vibration thresholds were 11.6 Hz higher in the neuropathy group compared to no neuropathy group and about 13.4 Hz higher when compared to the control group. The number of monofilament points detected was one unit less in the neuropathy group than the no neuropathy group as well as the control group. The peroneal nerve amplitudes were reduced by 1.8 mV in the neuropathy group compared to the other two groups. The peroneal nerve conduction velocities were reduced by 5 m/s in the neuropathy group and reduced by 7 m/s when compared to the control group.

Table 18. Clinical and neuropathy measures in the groups stratified per NDS criteria and in control group

Clinical variables and neuropathy measures	Neuropathy per NDS (A) Mean ± SD n Min - Max	No neuropathy per NDS (B) Mean ± SD n Min - Max	Controls (C) Mean ± SD n Min - Max	ANOVA *F*	ANOVA *p*-values	Bonferroni *p*-values
Total cholesterol (mmol/L)	4.3 ± 1.1 44 2.6 - 7.4	4.4 ± 0.9 106 2.8 - 8.6	5.6 ± 1.0 44 4.2 - 9.2	25.18	<0.001 *	C vs. A,B, <0.001
BP resting systolic (mmHg)	133 ± 14 44 101 - 166	126 ± 15 107 87 - 167	126 ± 14 44 102 - 163	4.74	0.010 §	A vs. B, 0.011 C vs. A, 0.04
BP resting diastolic (mmHg)	77 ± 8 44 60 - 97	76 ± 8 107 52 - 93	79 ± 9 44 63 - 108	1.28	0.279	
QST cold sensation threshold average (°C)	21.6 ± 9.5 44 0.0 - 31.1	26.0 ± 5.9 107 2.1 - 31.3	28.2 ± 2.7 44 19.6 - 33.2	11.46	<0.001 §	A vs B,C, 0.001
Peroneal M amp ankle to EDB (millivolts)	2.5 ± 2.0 44 0.1 - 9.6	4.3 ± 2.5 106 0.1 - 14.1	4.2 ± 2.5 44 0.1 - 9.2	8.19	<0.001 §	A vs. B, <0.001 A vs. C, 0.004
Peroneal CV ankle to FH (meters/second)	40.2 ± 6.5 44 20.0 - 52.5	45.2 ± 5.2 106 31.0 - 57.1	47.5 ± 7.4 44 20.0 - 59.0	16.60	<0.001 §	A vs. B, <0.001 A vs. C, 0.001

BP, blood pressure; QST, quantitative sensory testing; amp, amplitude; EDB, Extensor digitorum brevis; CV, conduction velocity; FH, Fossa head
* Significant differences between both diabetic groups versus control group with Tukey's HSD
§ Significant differences between neuropathy versus the other two groups with Tukey's HSD
Controls - no diabetes/neuropathy

Table 19. Descriptive statistics for clinical and neuropathy variables in the groups stratified per NDS criteria and in control group using non-parametric tests

Clinical and neuropathy variables	Neuropathy per NDS Median IQR Min - Max	No Neuropathy per NDS Median IQR Min - Max	Controls Median IQR Min - Max	Kruskal-Wallis test		
				χ^2	df	*p*-values
BMI (kg/m^2)	31.3 9.7 21 - 55	28.2 5.4 19 - 44	26.9 4.3 19 - 46	23.62	2	<0.001 ǂ
DNSS	1 3 0 - 4	0 1 0 - 4	0 0 0 - 1	31.32	2	<0.001 ǂ
QST warm sensation (°C)	43.1 8.2 32.8 - 50.0	38.3 5.9 19.4 - 49.2	37.2 6.5 30.5 - 45.4	22.76	2	<0.001 *
QST warm induced pain threshold (°C)	50 1.5 45.1 - 50.0	48.5 3.8 37.3 - 50.0	48.7 4.0 41.2 - 50.0	14.53	2	0.001 *
QST cold induced pain threshold (°C)	2.7 15.7 00.0 – 24.5	9.7 18.8 0.0 - 29.3	7.9 17.9 0.0 - 29.4	4.92	2	0.085
QST vibration threshold (Hz)	19.4 25.2 1.0 - 130.0	7.8 9.1 1.1 - 51.1	6.0 7.6 1.2 - 42.2	30.73	2	<0.001 ǂ
Monofilament (no. of points detected out of 3)	2 2 0 - 3	3 0 0 - 3	3 0 2 - 3	57.25	2	<0.001 *

BMI, body mass index; DNSS, diabetic neuropathy symptom score; QST, quantitative sensory testing;

ǂ Significant differences between any two groups with Mann-Whitney U test

* Significant differences between neuropathy versus the other two groups with Mann-Whitney U test

Controls - no diabetes/neuropathy

4.3.5.3 Full retinal thickness in the groups stratified by NDS and in the control group

Results are presented in Table 20. The full retinal thickness analysis showed that the thickness within the central 1mm zone was not significantly different in the neuropathy group compared to the other two groups. The overall thickness, the superior and inferior hemisphere thicknesses in the perifovea was significantly lower in individuals with neuropathy compared to those without neuropathy. The mean difference in thickness was about 7 μm. When compared to control group, those in the neuropathy group had significantly lower retinal thickness in the parafovea (9-11 μm) and in perifovea (7-8 μm) except the inferior perifovea. Figure 26 shows full retinal thickness in the groups stratified per NDS criteria and in the control group.

Table 20. Full retinal thickness in the groups stratified by NDS and in control group

Full retinal thickness (μm)	Neuropathy per NDS (A) Mean ± SD Min - Max	No neuropathy per NDS (B) Mean ± SD Min - Max	Controls (C) Mean ± SD Min - Max	ANOVA *F*	ANOVA *p*-values	Bonferroni *p*-values
Thickness within central 1mm zone (that includes fovea)	246 ± 19 207 - 286	245 ± 26 140 - 293	254 ± 26 180 - 313	2.30	0.102	
Overall parafovea	304 ± 14 271 - 330	310 ± 17 259 - 342	314 ± 14 277 - 341	4.54	0.012 †	A vs. C, 0.009
Superior hemisphere parafovea	307 ± 14 274 - 332	313 ± 17 254 - 349	318 ± 14 285 - 341	4.94	0.008 †	A vs. C, 0.006
Inferior hemisphere parafovea	302 ± 14 262 - 328	307 ± 17 246 - 343	311 ± 14 269 - 342	3.67	0.027 †	A vs. C, 0.024
Overall perifovea	264 ± 13 233 - 289	271 ± 15 201 - 301	272 ± 13 236 - 301	4.12	0.018 †‡	A vs. B 0.024
Superior hemisphere perifovea	270 ± 15 234 - 297	277 ± 15 228 - 313	278 ± 13 242 - 306	3.74	0.025 †‡	A vs. C, 0.040
Inferior hemisphere perifovea	258 ± 15 207 - 285	265 ± 16 170 - 302	265 ± 16 222 - 312	3.28	0.040 ‡	A vs. B 0.039

† Significant differences between neuropathy and control groups with Tukey's HSD
‡ Significant differences between neuropathy and no neuropathy groups with Tukey's HSD
Controls - no diabetes/neuropathy

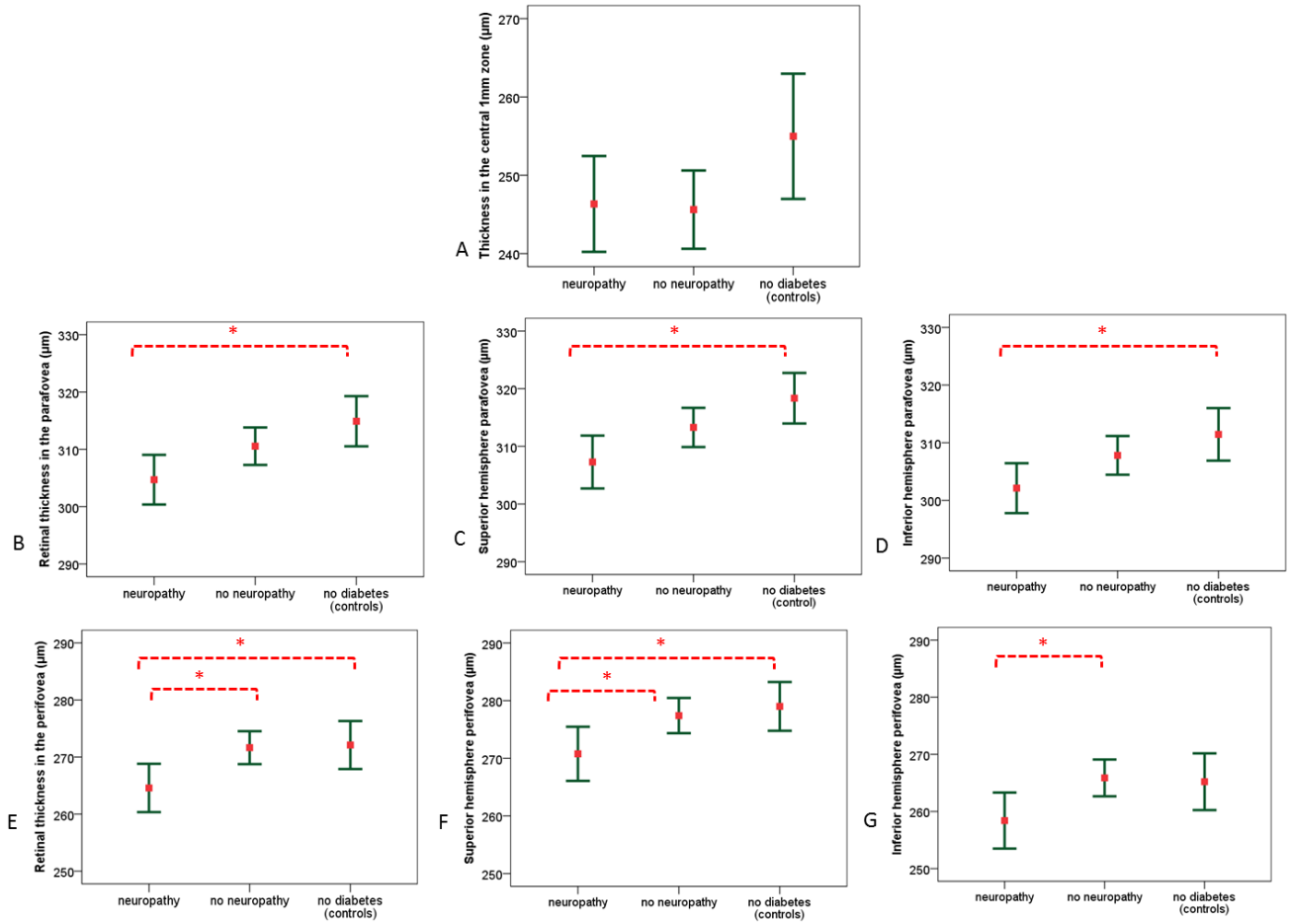


Figure 26. Full retinal thickness in the groups stratified per NDS and in control group for (A) central 1mm zone (B) overall parafovea (C) superior hemisphere parafovea (D) inferior hemisphere parafovea (E) overall perifovea (F) superior hemisphere perifovea (G) inferior hemisphere perifovea. Marker indicates mean retinal thickness in µm. Error bars are 95% CI. Asterisk represents significant difference

Retinal thickness was reduced in the perifovea in individuals with neuropathy compared to those without neuropathy. However, about 61% of participants with neuropathy had DR; therefore the relative effect of these variables on the retinal tissue thickness was explored.

Relationship between full retinal thickness and NDS, DR status, age, sex, duration of diabetes and HbA_{1c} levels in individuals with diabetes

Regression was performed in the group of individuals with diabetes. In the regression models, NDS was included as a continuous variable from 0-10.

Full retinal thickness and neuropathy by NDS criteria

The reduced overall and hemisphere thickness in the perifovea was significantly related to the severity of neuropathy (ranged from 1.06 μm to 1.58 μm). In the inferior perifovea, for every unit increase in NDS score, retinal thickness reduced by 1.58 μm and this was not significantly related to age, sex, DR, duration of diabetes or the HbA_{1c} levels. Neither the thickness in central zone ($p \geq 0.436$) nor that in parafovea ($p \geq 0.194$) showed any significant relationship to NDS. Results are presented in Table 21.

Relationship between full retinal thickness and other variables

The overall as well as hemisphere retinal thickness in the parafovea reduced by 7 μm to 8 μm, in the presence of DR; neither the thickness in the perifovea nor that in the other regions were significantly related to DR ($p \geq 0.055$).

Thickness within the central 1mm zone was about 10 μm greater in males when compared to females. The inferior parafovea showed a tendency for greater thickness in males than females and it approached statistical significance ($p = 0.051$). Other retinal regions did not differ significantly between males and females ($p \geq 0.076$).

The thickness within the central 1mm zone, in the overall and the hemisphere parafovea and perifovea reduced with increasing age, except the inferior perifovea ($p = 0.077$).

Neither the duration of diabetes ($p \geq 0.134$) nor the HbA_{1c} levels ($p \geq 0.624$) showed any significant relationship to full retinal thickness. Results are presented in Table 21.

Table 21. Relationship between full retinal thickness and NDS, age, sex, DR, duration of diabetes and HbA_{1c} levels in individuals with diabetes

	age			males			HbA_{1c} levels			duration of diabetes			DR			NDS			adjusted
	SE	B	p-value	SE	B	p-value	SE	B	p-value	SE	B	p-value	SE	B	p-value	SE	B	p-value	R^2
Thickness in the central 1mm zone	0.207	-0.529	**0.012**	3.899	10.222	**0.010**	0.153	0.158	0.915	3.934	0.230	0.134	1.475	-4.320	0.274	0.885	0.692	0.436	0.068
overall parafovea	0.137	-0.455	**0.001**	2.575	4.603	0.076	0.102	-0.074	0.940	2.586	0.045	0.660	0.979	-7.554	**0.004**	0.578	-0.649	0.264	0.100
superior hemisphere parafovea	0.144	-0.463	**0.002**	2.718	4.060	0.137	0.107	0.212	0.836	2.721	0.034	0.751	1.026	-6.880	**0.012**	0.609	-0.795	0.194	0.085
inferior hemisphere parafovea	0.139	-0.447	**0.002**	2.618	5.146	0.051	0.104	-0.360	0.720	2.626	0.056	0.591	1.003	-8.221	**0.002**	0.589	-0.502	0.395	0.102
overall perifovea	0.132	-0.322	**0.016**	2.407	0.607	0.801	0.093	0.439	0.624	2.519	0.040	0.669	0.895	-2.142	0.397	0.506	-1.170	**0.022**	0.086
superior hemisphere perifovea	0.136	-0.458	**0.001**	2.547	0.663	0.795	0.098	0.416	0.657	1.014	0.035	0.724	0.930	1.014	0.707	0.539	-1.060	0.051	0.083
inferior hemisphere perifovea	0.150	-0.268	0.077	2.728	2.646	0.334	0.107	0.497	0.630	2.864	0.043	0.687	1.031	0.295	0.055	0.555	-1.583	**0.005**	0.060

DR, diabetic retinopathy; NDS, neuropathy disability score; SE, standard error

The next section discusses the inner retinal layer thickness in relation to neuropathy per NDS criteria.

4.3.5.4 Inner retinal thickness in the groups stratified by NDS and in the control group

Results are presented in Table 22. Individuals with neuropathy had significantly reduced overall and the hemisphere RNFL thicknesses when compared to those without neuropathy when stratified by NDS criteria. The reduction in RNFL thickness ranged from 6-8 μm. However, the difference in GCC thickness was statistically significant (p = 0.049); however, post-hoc group comparisons did not reach statistical significance. Figure 27 shows RNFL and GCC thicknesses in the groups stratified per NDS criteria and in control group.

Table 22. Inner retinal thickness in the groups stratified by NDS and in control group

Inner retinal thickness (μm)	Neuropathy per NDS (A) Mean ± SD Min - Max	No neuropathy per NDS (B) Mean ± SD Min - Max	Controls (C) Mean ± SD Min - Max	ANOVA *F*	ANOVA *p*-values	Bonferroni *p*-values
Overall RNFL	97 ± 9 71 - 120	104 ± 11 75-136	102 ± 11 74-132	6.30	0.002 ‡	A vs. B 0.002
Superior hemisphere RNFL	98 ± 10 75 - 119	104 ± 12 76 - 136	102 ± 13 66 - 135	3.71	0.026 ‡	A vs. B 0.021
Inferior hemisphere RNFL	97 ± 9 75 - 119	105 ± 12 74 - 137	102 ± 12 82 - 136	7.26	0.001 ‡	A vs. B 0.001
Overall GCC	93 ± 7 77 - 112	96 ± 8 71 - 117	96 ± 7 76 - 108	2.63	0.075	
Superior hemisphere GCC	93 ± 7 75 - 112	96 ± 8 70 - 118	95 ± 8 70 - 110	2.18	0.115	
Inferior hemisphere GCC	93 ± 8 71 - 112	97 ± 8 73 - 118	97 ± 6 81 - 108	3.06	0.049 *	

‡ Significant differences between neuropathy and no neuropathy groups with Tukey's HSD
* No significant differences with post-hoc analysis in any group comparisons with Tukey's HSD
Controls - no diabetes/neuropathy

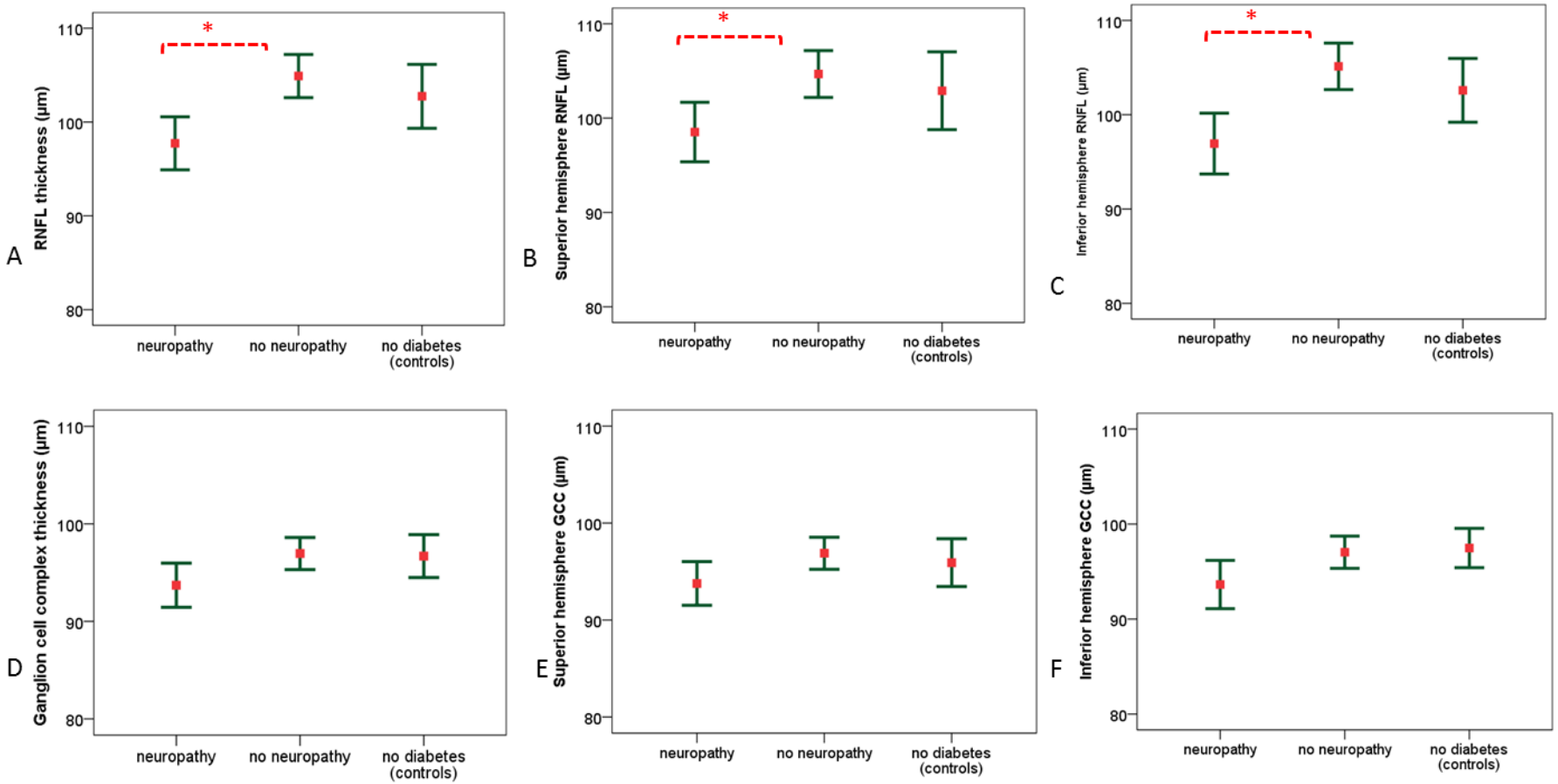


Figure 27. Inner retinal thickness in the groups stratified per NDS criteria and in control group for (A) overall RNFL (B) superior hemisphere RNFL (C) inferior hemisphere RNFL (D) overall GCC (E) superior hemisphere GCC (F) inferior hemisphere GCC. Marker indicates mean inner retinal thickness in µm. Error bars are 95% CI. Asterisk represents significant difference

Relationship between inner retinal thickness and NDS, DR status, age, sex, duration of diabetes and HbA_{1c} levels in individuals with diabetes

In the regression models, NDS was included as a continuous variable from 0-10.

Inner retinal thickness and neuropathy per NDS

The overall as well as hemisphere RNFL thickness reduced with NDS or in other words, increasing severity of neuropathy, when adjusted for age, sex, DR, duration of diabetes and HbA_{1c} levels. For every unit increase in NDS, the RNFL thickness decreased by 1.204 µm, 0.851 µm and 1.448 µm in the overall, superior hemisphere and inferior hemisphere respectively. GCC thickness was not significantly related to neuropathy ($p \geq 0.065$).

Relationship between inner retinal thickness and other variables

The RNFL thickness and GCC thickness reduced with advancing age. Males with diabetes had significantly thinner RNFL in the inferior hemisphere than females ($p = 0.03$). Other regions did not show significant differences between males and females ($p \geq 0.178$).

Variables namely DR ($p \geq 0.367$), duration of diabetes ($p \geq 0.210$), HbA_{1c} levels ($p \geq 0.652$) were not significantly related to RNFL or the GCC thicknesses. Results are presented in Table 23.

Table 23. Relationship between inner retinal thickness and NDS, age, sex, DR, duration of diabetes and HbA_{1c} levels in individuals with diabetes

	age			males			HbA_{1c} levels			duration of diabetes			DR			NDS			adjusted
	SE	B	p-value	SE	B	p-value	SE	B	p-value	SE	B	p-value	SE	B	p-value	SE	B	p-value	R^2
overall RNFL	0.098	-0.325	**0.001**	1.779	2.406	0.178	0.066	-0.062	0.928	1.982	0.083	0.210	0.679	0.403	0.839	0.375	-1.204	**0.002**	0.161
superior hemisphere RNFL	0.108	-0.357	**0.001**	2.011	0.821	0.684	0.073	-0.331	0.652	2.226	0.085	0.242	0.733	0.911	0.683	0.413	-0.851	**0.041**	0.114
inferior hemisphere RNFL	0.106	-0.293	**0.006**	1.916	-4.203	**0.030**	0.071	0.284	0.694	1.965	0.078	0.275	0.721	-0.238	0.912	0.405	-1.448	**<0.001**	0.186
overall GCC	0.070	-0.271	**<0.001**	1.352	0.055	0.967	0.052	0.124	0.803	1.394	0.010	0.856	0.496	-1.156	0.408	0.280	-0.520	0.065	0.085
superior hemisphere GCC	0.070	-0.293	**<0.001**	1.333	-0.039	0.977	0.052	0.088	0.860	1.388	0.032	0.541	0.500	-0.965	0.488	0.278	-0.501	0.074	0.100
inferior hemisphere GCC	0.075	-0.250	**0.001**	1.444	-0.200	0.890	0.055	0.170	0.747	1.484	-0.007	0.906	0.527	-1.343	0.367	0.298	-0.539	0.073	0.064

RNFL, retinal nerve fibre layer; GCC, ganglion cell complex; DM, diabetes mellitus; DR, diabetic retinopathy; NDS, neuropathy disability score; SE, standard error

4.3.6 Discussion

Full retinal thickness

This research project examined for the relationship between diabetic neuropathy and retinal tissue thickness. The main finding was that the retinal thickness in the perifovea or in other words, the outer macula, reduced in relation to NDS scores or severity of neuropathy.

The increasing severity of neuropathy was associated with reduced thickness in the perifovea but not in the parafovea. Diabetic peripheral neuropathy involves distal nerves first and then progresses to involve proximal nerves (e.g. in the legs) (Brown et al., 1980). With reference to the central zone, perifovea is distal or peripheral whereas parafovea is relatively proximal, indicating distal or more peripheral regions of retina affected. This appears similar to diabetic peripheral neuropathy, wherein, the distal nerves are affected first and then the proximal nerves are involved in later stages.

Another likely explanation could be that the findings in perifovea may represent changes in the outer retinal layers as the perifovea mostly comprises of RPE and photoreceptors (Sernagor et al., 2001). Since neuropathy predominantly involves damage to the sensory receptors in the distal leg, changes in the perifovea may represent involvement of photoreceptors in the retina in relation to neuropathy. However, this supposition necessitates further investigation.

The thickness in the perifovea was reduced with increasing severity of neuropathy and advancing age while that in the inferior perifovea was reduced with severity of neuropathy only. Another study reported significantly reduced multifocal visually evoked potential (mfVEP) amplitudes in the lower nasal retinal quadrant in people with neuropathy when compared to people without neuropathy (Lövestam-Adrian et al., 2012). A relatively common observation is that the inferior retinal region appears to be specifically compromised in relation to diabetic neuropathy. A likely explanation for this disturbance to the inferior retinal region could be the lower blood flow per nerve fibre tissue

volume in the normal human retina in the inferior region (Harris et al., 2003) and may be more prone to ischaemic insults; this effect may be exaggerated in diabetes.

To date, this is the first study to examine the relationship between diabetic peripheral neuropathy and full retinal thickness; the reduced thickness in the perifovea was related to the severity of neuropathy. The magnitude of this difference in retinal thickness may appear smaller (1.06 -1.58 μm); however, this observed effect is after adjusting for potential confounding factors namely age, sex, DR, HbA_{1c} levels and duration of diabetes. A likely explanation for the apparently small effect may be that 62% of individuals had mild neuropathy (NDS 3-5), 30% of individuals had moderate neuropathy (NDS 6-8) and only about 8% with severe neuropathy (NDS 9-10) based on the neuropathy severity grading proposed by Young et al (1993). Another explanation for the apparently small effect could be that the results demonstrate an association that is between local responses in the retina and neuropathy changes in the distal leg. Interestingly, after stratifying groups based on NDS criteria, retinal thickness was reduced by 7 μm; this resembles an age-related change of 25-40 years. In addition, in individuals without diabetes, retinal thickness decreased by 0.26-0.46 μm, according to Ericksson et al (2009) and by 0.53 μm, according to Alamouti et al (2003) for a year increase in age. In the current study, with regression analysis, this reduction or compromise in relation to neuropathy was 1.2 μm (1.06-1.58 μm), which is 5-7 times higher when compared to these reported age-related changes. Although the absolute effect of neuropathy on retinal thickness may appear small, it essentially reflects a physiologically relevant change.

Diabetic retinopathy is a frequently reported factor associated with altered retinal tissue thickness. In the current study, thickness in the parafovea was reduced in the presence of DR. Previous studies reported differing results. For instance, individuals with any stage of DR had greater retinal thickness (Goebel, et al., 2002) and a thicker central 2x2 mm^2 area compared to those without diabetes (Oshima, et al., 1999); 30% of eyes with NPDR had greater thickness compared to those without diabetes in at least two ETDRS zones (Massin et al.,

2002). A trend of increase in macular thickness with increasing severity of DR has also been observed with a vertical scan through the fovea (Cho et al., 2010) thicker macula (Lattanzio, et al., 2002; Park, et al., 2011). Few other studies observed similar thicknesses in the groups without DR (Massin et al., 2002) and with DR (Bressler et al., 2008; Cho, et al., 2010) when compared to those without diabetes. In contrast, few other studies observed reduced thickness in the parafovea in individuals with no DR (Nilsson, et al., 2007) and reduced overall macular thickness (Oshitari, et al., 2009) in comparison to those without diabetes. The above studies differed in terms of their study objectives, scan protocols, the age group examined, differences in male-female composition and showed disparity in the consideration of factors such as the duration of diabetes and HbA_{1c} levels. Also the neuropathy status of the participants was not taken into account. The current research program has utilized regression models and has accounted for the key variables namely age, sex, DR, duration of diabetes and HbA_{1c} levels and neuropathy defined by NDS.

In the current study, there were 63 (42%) individuals with DR and 88 (58%) individuals with diabetes but no DR. Amongst those with DR, the majority (88%) of individuals had very mild NPDR or mild NPDR and very few individuals with more advanced stages of DR. However, investigating retinal tissue thickness in varying severity of DR was not the primary focus of this research program. For this reason, the individuals with DR were grouped as one and presence of DR rather than the severity of DR were accounted for in the statistical models. It is relevant to note that diabetic retinopathy here represents only the clinically visible signs of retinopathy. There may be sub-clinical or subtle retinopathy changes that are not clinically visible. The possibility of subtle retinopathy changes being related to any of the outcomes could not be ruled out and therefore requires caution when interpreting the results.

Findings from the current study show that retinal thickness decreased with advancing age; an exception to this being the inferior perifovea which was significantly related to the severity of neuropathy but not with age. Previous studies reported reduction in retinal thickness with advancing age in

individuals without diabetes (Alamouti et al., 2003, Fraser-Bell et al., 2005, Eriksson et al., 2009). In contrast, studies conducted on individuals with diabetes observed increase in central thickness with age in people with diabetes with or without DR (Sánchez-Tocino et al., 2002, Fritsche et al., 2002). However, the individuals with diabetes were much older than the group without diabetes, with a difference as high as 29 years in the study by Fritsche and his group; therefore, the possibility of confounding due to age could not be ruled out. In addition, diabetic neuropathy was not taken into account. The current study adjusted for age, neuropathy status and other potential confounders.

The thickness in the central zone was greater in males with diabetes than females with diabetes, when adjusted for age, DR, HbA_{1c} levels and duration of diabetes. Previous studies observed greater thickness in the central zone and higher total macular volume in males with diabetes than females (Browning et al., 2008). Another study observed that the males in the diabetic group who did not show clinical signs of DR had 12 μm thicker retina than the females (Oshitari et al., 2009); a between-gender difference of 14 μm was observed in individuals without diabetes.

Although it is likely that the retinal thickness may be compromised in relation to prolonged duration of diabetes, glycaemic level is an important but also a modifiable risk factor. A previous study observed that the thickness in the macular region was inversely correlated to HbA_{1c} levels (Moon et al., 2011). Other studies observed reduced visual function demonstrated by delayed P100 latency of visual evoked potentials (Tobimatsu et al., 1991) in young people with diabetes without DR, which normalized following optimal metabolic control (Verrotti et al., 2000). In the current study, HbA_{1c} levels were not significantly related to full retinal thickness when adjusted for duration of diabetes and other factors. This is consistent with a study by Asefzadeh et al (2008) who observed no significant relationship between retinal thickness and HbA_{1c} levels. Although, Moon et al observed that the thickness in the macular region to be influenced by HbA_{1c} levels, the relationship was assessed using correlations. The current research program used regression models to adjust for potential confounding variables. The lack of significant relationship to HbA_{1c}

levels may be explained by the following; higher glycaemic levels have been reported to be related to development and progression of neuropathy (Tesfaye et al, 1996) and retinopathy (Diabetes Control and Complications Trial, 1995); however, a strict (more than 2% reduction) and an acute or rapid reduction in HbA_{1c} levels were associated with worsening of DR (Funatsu et al., 1992) (Davis, 1998); also after a 4 month-strict metabolic control, the RNFL thickness reduced further compared to baseline values (Sugimoto et al., 2010). The findings reported in previous studies and that from this study may indicate (i) a non-linear relationship in retinal changes in response to alterations in HbA_{1c} levels (ii) the extent or the percentage of reduction in glycaemic levels is equally important, in addition to the absolute values of HbA_{1c} levels. The current study was cross-sectional; therefore, the previous HbA_{1c} levels were not recorded.

Inner retinal thickness

The RNFL thickness was reduced in individuals with neuropathy compared to those without neuropathy; the reduced thickness was related to diabetic peripheral neuropathy when adjusted for DR status, sex, duration of diabetes and HbA_{1c} levels. The difference in GCC thicknesses did not reach statistical significance. To date, this is the first study to investigate the relationship between macular inner retinal thickness (GCC) and neuropathy in a group of individuals with diabetes.

The individuals with diabetic neuropathy were significantly older (mean = 4.5 years) than individuals without neuropathy; the unadjusted group difference in RNFL thickness ranged from 8 to 9 μm. Given that the RNFL thickness decreases by 2 μm for a decade increase in age (Bundez et al., 2007), the difference noted in the current study was 8-9 μm for a 4.5 years difference in age; this decrease in thickness is greater than that expected due to age alone. Another way of looking at this is that people with neuropathy have neural changes that are similar to that seen in an individual 40-45 years older.

In the current study, GCC thickness did not show significant relationship to neuropathy. The retinal nerve fibre layer thickness reduced with increasing

severity of neuropathy by 0.86 μm to 1.23 μm. Other variables such as HbA_{1c} levels, duration of diabetes and retinopathy failed to explain the findings. The previous pilot study (Shahidi et al., 2012) that investigated the RNFL thickness in relationship to diabetic neuropathy observed inferior RNFL thickness to reduce by 1.5 μm for a unit increase in NDS in a group of individuals with type 2 diabetes, when adjusted for age, duration of diabetes and retinopathy. The RNFL findings from the current study are not surprising, as the pilot study by Shahidi et al (2012) was expanded further. The current project took a comprehensive approach and examined the full retinal thickness, macular and the peripapillary nerve fibre layer thicknesses, in relation to diabetic peripheral neuropathy, in a higher sample size.

Another interesting finding is that DR did not influence the RNFL or GCC thickness when neuropathy and other factors were taken into account. This is again in agreement with that reported by Lövestam-Adrian et al (2012) who also found retinal anatomical and functional changes in relation to neuropathy but not with DR. Degeneration of ganglion cell layers and inner plexiform layer and thinning of RNFL was a common observation in both, the presence (Wolter, 1961, Bloodworth, 1962, Takahashi et al., 2006, Oshitari et al., 2009, van Dijk et al., 2009, van Dijk et al., 2010) and the absence of clinical signs of DR (Sugimoto et al., 2005, Peng et al., 2011, Verma et al., 2012,). In those studies, neuropathy may have been a contributing factor to the compromised neural layer thicknesses. In the current study, the GCC as well as the RNFL thickness, reduced with advancing age. This finding is consistent with that reported in previous studies (Bundez et al., 2007, Koh et al., 2012).

In the current study, it was observed that the inferior RNFL thickness is about 4 μm thinner in males in the group with diabetes compared to females when adjusted for other factors. Males with diabetes had a thicker inferior parafovea and a thinner inferior hemisphere RNFL. In contrast, in individuals without diabetes, Koh et al (2012) observed the ganglion cell complex-inner plexiform layer thickness to be thinner in females compared to males. In the current research program, the data did not appear to be influenced by any outliers. For that matter, this observation is also true for the control group, where in, males

showed a tendency for reduced RNFL thickness compared to females. Literature reports that there is a male preponderance in diabetic retinopathy (Bodansky et al., 1982; Zhang et al., 2010) and diabetic neuropathy (Booya et al., 2005). Therefore, the gender difference in the current study could be related to retinopathy or neuropathy. However, retinopathy did not show significant relationship to RNFL or the GCC thickness. Therefore, this gender difference in RNFL is less likely to be related to retinopathy. Interestingly, the RNFL thickness was significantly reduced in neuropathy. In addition, it is noteworthy that the proportion of males with neuropathy was higher (68%) compared to those without neuropathy (54%) though not statistically significantly (p =0.114). Therefore, this gender differences in RNFL may be linked to neuropathy. These findings demonstrate differences between males and females with respect to retinal region examined. However, this observation will require further exploration.

In the current study, neither the retinal nerve fibre layer nor the GCC thickness showed any significant relationship to duration of diabetes or the HbA_{1c} levels when including NDS, DR status, age and sex of the individuals into the models. A previous study observed a higher proportion of people with neuropathy among those with longer duration of diabetes (Tesfaye et al., 1996). Interestingly, treatment of chronic hyperglycaemia was associated with improvement in neuronal function thus indicating a strong association with HbA_{1c} levels (Boulton, 1982) and also the potential for neuropathy status to improve with intervention. Because of the cross-sectional design of the current study, previous HbA_{1c} levels were not monitored. However, when including these factors into the statistical models, thickness differences in inner retinal layers was significantly related to peripheral neuropathy rather than the HbA_{1c} levels or the duration of diabetes.

Lack of significant relationship between neuropathy and GCC thickness may indicate the following (i) as the axons (representing the RNFL) from all over the retina converge at the optic nerve head, the changes happening in the axons of the retinal nerves (RNFL) may be more easily detectable around the optic nerve head as it purportedly represents a global measure of the RNFL thickness (ii)

loss in the nerve cell bodies (ganglion cells) outside the 7 mm x 7 mm zone, if any, may have been missed by GCC protocol. This supposition is further strengthened by the observation that the relationship with GCC thickness was close but did not reach statistical significance.

4.3.7 Conclusion

Severity of neuropathy is associated with reduced thickness in the perifovea or the outer macula and the peripapillary nerve fibre layer. Retinopathy is associated with compromised thickness in the parafovea (inner macula). It appears that neuropathy and retinopathy involve different retinal regions.

The inner retinal thickness was reduced in relation to diabetic peripheral neuropathy but did not show significant relationship to retinopathy, thus demonstrating that the neuroretinal degeneration in diabetes is significantly related to peripheral neuropathy rather than retinopathy. However, these are preliminary findings only and require further investigation.

While the inner retinal layers comprise of neural elements that play a vital role in vision, a reduction in retinal layer thicknesses have important implications for a potential compromise in visual function. Future studies are indicated to examine the retinal tissue thickness and assess the visual function in people with neuropathy and also to investigate in individuals with mild versus more severe degrees of neuropathy. This will provide a better understanding of the associated visual implications for individuals with diabetic peripheral neuropathy.

Chapter 5: Pattern-based ganglion cell complex parameters in relation to diabetic neuropathy

5.1 Background

Results thus far from the current project demonstrate compromised full retinal thickness, RNFL thickness in relation to diabetic peripheral neuropathy. The GCC thickness showed a tendency to be reduced however this relationship did not reach statistical significance.

It is interesting to note that in addition to the GCC thickness profile, there is also a pattern to this GCC loss. Previous studies in glaucoma (Tan et al., 2009) and macular degeneration (Garas et al., 2012) models have observed that this pattern-based GCC loss, namely focal and global loss in GCC volume, can detect more abnormalities than the GCC thickness profile. This provides a good platform to investigate for this loss in relation to diabetic peripheral neuropathy. Therefore, this part of the study investigated the pattern-based GCC parameters, namely the focal and global loss in GCC volume, in relation to diabetic peripheral neuropathy. It was expected that this part of the analysis will contribute to new knowledge regarding structural integrity of the inner retina at the macular area in relation to diabetic neuropathy.

With the use of OCT, one can reportedly detect focal loss in ganglion cell volume (FLV) and global loss in ganglion cell volume (GLV) derived in comparison to population norms. The following section presents an overview of the calculation of FLV and GLV parameters.

Calculation of GLV and FLV

The ganglion cell complex thickness represents thickness from the inner plexiform layer to the nerve fibre layer, centred at the macula. Figure 28 shows

(A) the thickness map (B) the deviation map and (C) the significance map for a participant.

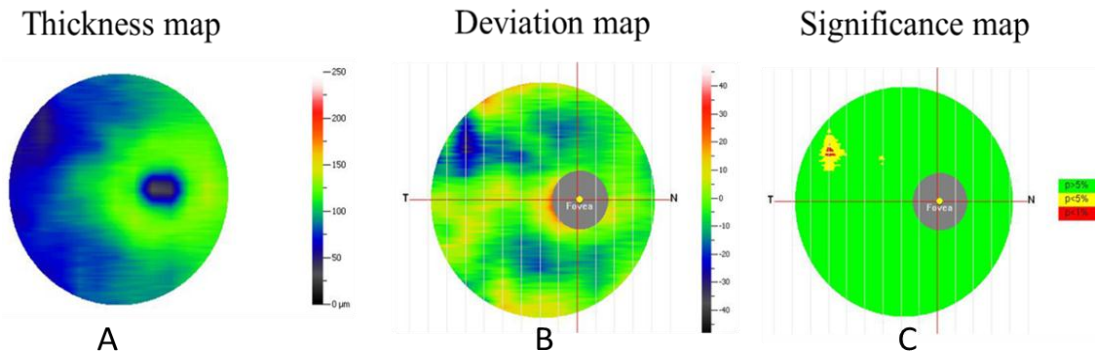


Figure 28. Ganglion cell complex maps (A) the GCC thickness map (B) the GCC deviation map (C) the GCC significance map. The FLV and GLV are quantitative parameters estimated from these maps.

The thickness map in Figure 28A displays thickness values at each pixel. Warmer colours represent thicker regions while cooler colours represent thinner regions. A deviation map, on the other hand, is derived by comparing each pixel with that of age-matched map from that of the general population. On a deviation map, cooler colours represent the thinner regions relative to the general population. The scale on the right side of the deviation map in Figure 28 B shows pixels below 0; this represents thickness that is relatively lower in comparison to that of age-matched map from general population. From these maps, the GLV and FLV are calculated.

The GLV indicates global loss in GCC volume over the entire GCC map. The percentage decrease in thickness at each pixel in comparison to the normative database is displayed on a map called the fractional deviation map. The pixels with values below 0 on this fractional deviation map are summed up and divided by the entire GCC area to provide the GLV.

The FLV indicates focal loss in GCC volume over the entire GCC map. The thickness value at each pixel is compared to that of population norm to give a pattern map. This map for the participant is then compared with the average pattern map from the age-matched database. The difference between the two maps is the pattern deviation map, which is assigned significance values for the

deviation. The pixel values less than '0' in the fractional deviation map are summed along with those from the pattern deviation map that have loss at < 5% level and divided by the total area, to provide the FLV.

Figure 29 shows GCC FLV and GLV parameters for another participant and the significance of this loss flagged. 'No significant loss' where points or regions are equal to or above 5% of the general population are represented by green. The measured value for the FLV or the GLV if outside the normal range, is highlighted in yellow or red. 'Borderline loss', for instance, is indicated if the measured value is below the 5% level when compared to that of the normative database, and the point or region is represented in yellow. Significance at < 5% indicates that one in 20 individuals in the general population will most likely exhibit this value or less. If the measured value is below the 1% level when compared to that of the general population, the point or region is represented in red indicating 'outside normal loss'. Significance at < 1% indicates that one in 100 individuals in the general population will most likely exhibit this value or less.

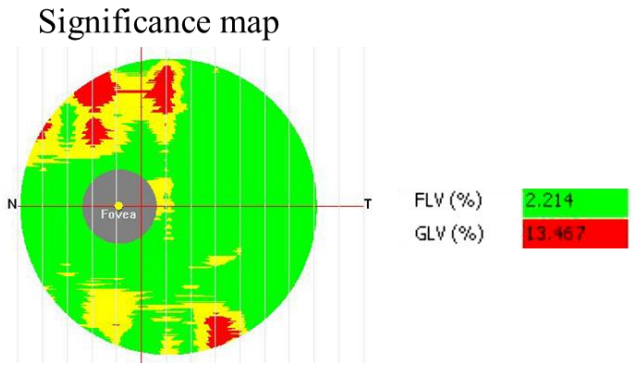


Figure 29. Significance map of another participant with greater abnormality. Significance of this loss is flagged. The FLV represented in green indicates 'not a significant loss' or probability > 5%; the GLV flagged in red indicates a significant loss at probability < 1%. This participant has 'within normal' FLV and an 'outside normal' GLV.

In a previous study, the frequency of borderline and outside normal loss in GCC FLV and GLV has been assessed in eyes with subfoveal choroidal neovascularisation in comparison to that of non-diseased eyes (Garas et al.,

2012). A greater proportion of eyes with subfoveal choroidal neovascularisation had significant focal loss in GCC volume than the non-diseased eyes. This provides a good platform to investigate this loss in relation to diabetic neuropathy. Therefore, this study sought to explore the focal and global loss in GCC volume, and the proportion of individuals with significant focal and global ganglion cell loss and the factors explaining this loss.

5.2 Purpose

The purpose of this study was to examine the focal and global loss in GCC volume in individuals with and without neuropathy, and also to compare with that of individuals without diabetes and without neuropathy (control group). The study also examined the proportion of individuals with significant focal and global loss in ganglion cell volume relative to population norms and the factors explaining the loss.

Research question

Are the focal and global losses in GCC volume significantly related to diabetic peripheral neuropathy?

5.3 Methods

The focal (FLV) and global (GLV) loss in GCC volume was examined in individuals with and without neuropathy defined by NDS criteria and in the control group. The FLV and GLV values are expressed in percentages. The proportion of individuals with borderline or outside normal limits loss in FLV and GLV was determined and compared to that of the control group. The outcome is represented as percentage.

The relationship between the loss in GCC volume and diabetic neuropathy was then examined in the group of individuals with diabetes, taking into account, the presence of retinopathy, age, sex of the individuals, duration of diabetes and HbA_{1c} levels.

5.4 Statistical analysis

The FLV and GLV did not follow a normal distribution. Therefore, the group differences in FLV and GLV were assessed using a Kruskal-Wallis test; a Mann-Whitney U test was performed for those with significant results on Kruskal-Wallis test. To determine if loss in GCC volume is related to neuropathy, a binary logistic regression was performed in the group of individuals with diabetes. Individuals with significant focal loss in GCC volume were coded as '1' and those with 'not a significant loss' were coded as '0' and were entered as dependent variable in the regression. Variables namely age, HbA_{1c} levels, duration of diabetes and NDS were entered as continuous variables; sex of the individuals and the presence of retinopathy were coded and entered as categorical variables. The models were then modified to obtain the most parsimonious model. The same procedure was followed for significant global loss in GCC volume.

5.5 Results

Table 24 presents the summary statistics and the group differences in FLV and GLV. Individuals with neuropathy had higher focal loss in GCC volume compared to those without neuropathy (p = 0.008), as well as when compared to that of the control group (p < 0.001); individuals in the no neuropathy group had higher FLV when compared to those in the control group (p = 0.044).

With regards to the GLV, individuals with neuropathy had higher global loss in GCC volume when compared to those without neuropathy (p = 0.033), as well as with that of the control group (p = 0.008); however, the GLV in the no neuropathy group did not differ significantly when compared to that of the control group (p = 0.406).

Table 24. Comparison of FLV and GLV in the neuropathy, no neuropathy and control groups

	Neuropathy Median IQR Min - Max	No neuropathy Median IQR Min - Max	No diabetes/ neuropathy(Controls) Median IQR Min - Max	Kruskal-Wallis		
				χ^2	df	*p*-value
GCC FLV (%)	0.97 2.99 0.00 - 6.18	0.41 1.43 0.00 - 5.78	0.24 0.67 0.00 – 3.87	15.57	2	< 0.001 §
GCC GLV (%)	5.38 7.99 0.02 – 21.48	3.36 6.11 0.00– 24.20	2.44 6.25 0.02 – 14.19	7.23	2	0.027 ‡

§ Significant difference between any two groups with Mann-Whitney U test
‡ Significant difference between neuropathy versus no neuropathy as well as the control group with Mann-Whitney U test

Focal loss in GCC volume

Table 25 shows the proportion of individuals with borderline and outside normal FLV loss when stratified according to neuropathy status as well as in the control group.

Table 25. Proportion of individuals with borderline or outside normal FLV loss stratified according to neuropathy status and in the control group

Number of participants with FLV significant loss at:	Neuropathy	No neuropathy	Controls
Borderline loss in FLV	1 (2.3%)	6 (5.7%)	0
Outside normal loss in FLV	10 (23.3%)	5 (4.8%)	2 (5%)
Total no. with 'significant FLV loss'	**11 (25.6%)**	**11 (10.5%)**	**2 (5%)**

About 25.6% of those with neuropathy, 10.5% of those with no neuropathy and 5% in the control group had significant focal loss in GCC volume. Figure 30 illustrates the proportion of individuals with FLV loss in the groups when stratified according to NDS criteria of neuropathy as well as in the control group. A chi-square test of independence was significant, $\chi^2_{(2,\ 188)} = 9.002$,

p = 0.011, and the proportion of people with the FLV loss was significantly higher in the neuropathy group than the other two groups.

The proportion in the control group is as expected when compared to the population norms. On the other hand, the group with diabetes show greater proportion of individuals with loss in comparison to population norms. Therefore, to determine the variable(s) explaining this loss, a regression analysis was performed only in the group with diabetes.

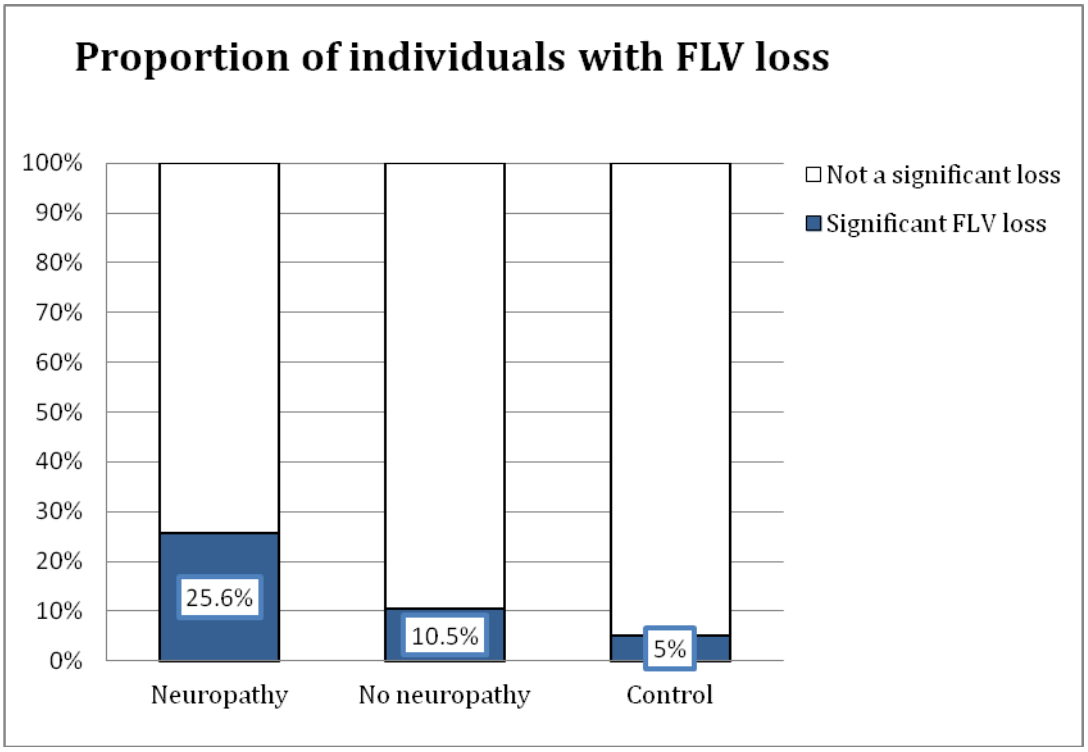


Figure 30. Proportion of individuals with FLV loss when stratified according to neuropathy status and in the control group. The series in blue represent those with 'significant FLV loss' (probability < 5% or < 1%)

The relationship between significant focal loss in GCC volume and NDS, DR, age, sex, duration of diabetes and HbA_{1c} levels, in individuals with diabetes

Variables entered in the model are presented in Table 26. NDS was entered as a continuous variable from 0-10. Diabetic retinopathy was entered as presence or the absence of DR. This model was then modified to obtain the most parsimonious model. The final model is presented in Table 27. With every unit increase in NDS, the odds of having a significant focal loss in GCC volume increased by a multiplicative factor of 1.249, 95% CI [1.063, 1.468]; this loss is not significantly related to DR status, age, sex, duration of diabetes or the HbA_{1c} levels.

Table 26. Variables entered in logistic regression. Presence or the absence of significant focal loss in GCC volume was entered as dependent variable

	B (SE)	Wald	df	*p*-value	Odds ratio
Constant	-0.665 (2.320)				
NDS	0.127 (0.096)	1.735	1	0.188	1.135
Age	0.004 (0.030)	0.017	1	0.895	1.004
Males	-0.255 (0.496)	0.436	1	0.509	1.410
Retinopathy	1.443 (0.570)	5.213	1	0.022	0.236
HbA_{1c}	-0.112 (0.192)	0.342	1	0.559	0.894
Duration of diabetes	-0.013 (0.022)	0.369	1	0.544	0.987

SE, standard error; NDS, neuropathy disability score

Table 27. Final regression model showing that significant focal loss in GCC volume is related to NDS, when adjusted for presence of DR, age, sex, duration of diabetes and HbA_{1c} levels

	B (SE)	Wald	df	*p*-value	Exp(B)	95% CI for Exp(B)	
						Lower	Upper
Constant	-2.277 (0.338)						
NDS	0.222 (0.082)	7.280	1	0.007	1.249	1.063	1.468

SE, standard error; NDS, neuropathy disability score

Global loss in GCC volume

Table 28 shows the proportion of individuals with borderline and outside normal GLV loss when stratified according to neuropathy status and in the control group.

Table 28. Proportion of individuals with borderline and outside normal GLV loss stratified according to neuropathy status and in the control group

Number of participants classified to have significant GLV loss at:	Neuropathy	No neuropathy	Controls
Borderline loss in GLV	3 (7%)	2 (1.9%)	0
Outside normal loss in GLV	3 (7%)	8 (7.6%)	1 (2.5%)
Total no. with 'significant GLV loss'	**6 (14%)**	**10 (9.5%)**	**1 (2.5%)**

For the GLV, 14% of individuals with neuropathy, 9.5% of those with no neuropathy and 2.5% of those in the control group had significant GLV loss. Figure 31 illustrates the proportion of individuals with GLV loss in the three groups. The chi-square test of independence was not significant, $\chi^2_{(2,\ 188)} = 3.372$, $p = 0.185$. In the control group, the proportion of individuals with this significant loss is as expected when compared to the population norms. On the other hand, those in the group with diabetes show greater proportion of individuals with loss when compared to population norms. Although, the analysis of this loss indicated no statistically significant differences when stratified according to NDS, this loss may be related to other variable(s). Therefore, to determine the variable(s) that may explain this loss, a regression analysis was performed in the group with diabetes.

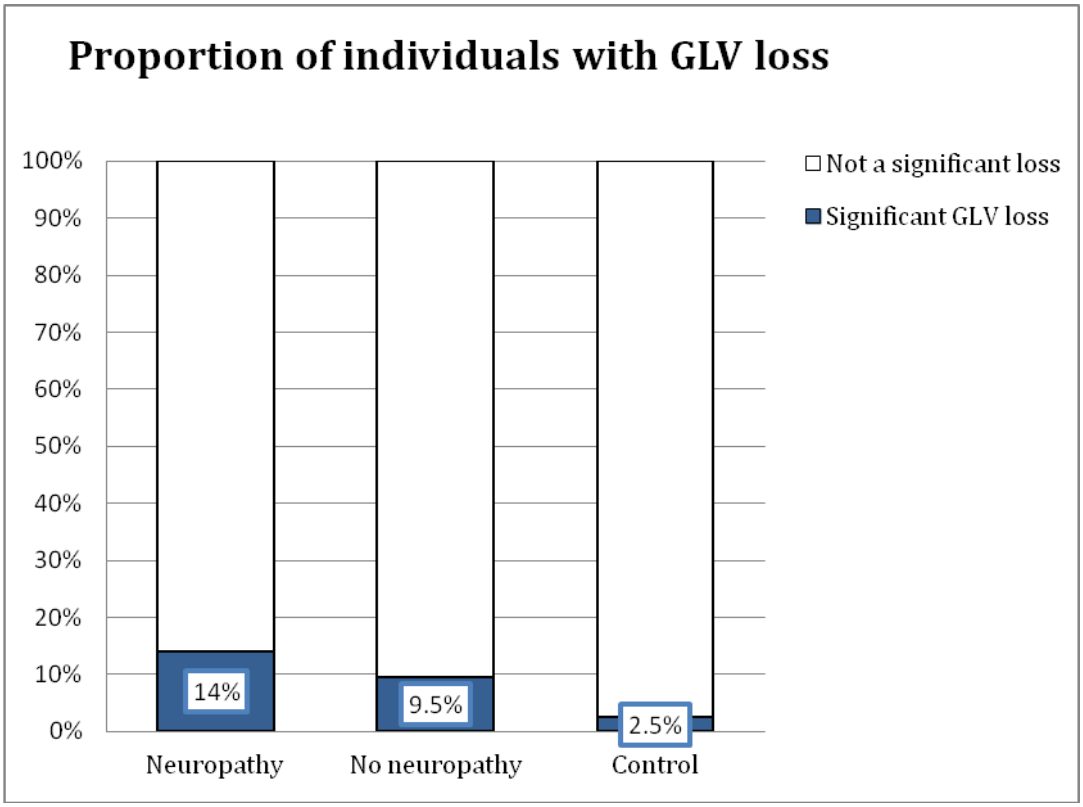


Figure 31. Proportion of individuals with GLV loss when stratified according to neuropathy status and in the control group. The series in blue represent those with 'significant GLV loss' (probability < 5% or < 1%)

The relationship between significant global loss in GCC volume and NDS, DR, age, sex, duration of diabetes and HbA_{1c} levels, in individuals with diabetes

The variables entered in the model are presented in Table 29. NDS was entered as a continuous variable from 0-10. This model was then modified to obtain the most parsimonious model. The final model is presented in Table 30. With every year increase in age, the odds of having a significant global loss in GCC volume increased by a multiplicative factor of 1.077, 95% CI [1.009, 1.149]; this loss was not significantly related to the severity of neuropathy, DR status, sex, duration of diabetes or the HbA_{1c} levels.

Table 29. Variables entered in logistic regression. Presence or the absence of significant global loss in GCC volume was entered as dependent variable

	B (SE)	Wald	df	*p*-value	Odds ratio
Constant	-5.785 (2.983)	3.76	1	0.053	0.003
NDS	0.083 (0.116)	0.506	1	0.477	1.086
Age	0.082 (0.039)	4.41	1	0.036	1.086
Males	-0.578 (0.612)	0.891	1	0.345	1.782
Retinopathy	0.683 (0.711)	0.923	1	0.337	0.505
HbA_{1c}	-0.211 (0.236)	0.797	1	0.372	0.81
Duration of diabetes	0.012 (0.023)	0.28	1	0.597	1.012

SE, standard error; NDS, neuropathy disability score

Table 30. Final regression model showing that significant global loss in GCC volume is related to advancing age, when adjusted for NDS, presence of DR, sex, duration of diabetes and HbA_{1c} levels

	B (SE)	Wald	df	*p*-value	Exp(B)	95% CI for Exp(B)	
						Lower	Upper
Constant	-6.448 (2.040)						
Age	0.074 (0.033)	4.932	1	0.026	1.077	1.009	1.149

SE, standard error;

5.6 Discussion

This part of the study sought to explore the research question, if the focal loss (FLV) and global loss (GLV) in GCC volume is significantly related to diabetic peripheral neuropathy. Results from this research project demonstrate pockets of ganglion cell loss in individuals with diabetes that was related to the severity of neuropathy; individuals with diabetes also had generalized ganglion cell loss that was related to advancing age. It is interesting to note that these results were not significantly related to diabetic retinopathy or other factors such as duration of diabetes, HbA_{1c} levels or sex of the individuals. Diabetic retinopathy did not show significant relationship to this loss, thus supporting the proposition that the previously reported visual field compromise (Stavrou et al., 2005; Kern et al., 2008; Parravano et al., 2008) may be related to neural pathology elsewhere in the body in diabetes.

The odds ratio for both FLV and GLV indicate that there is an association, although the lower limit of confidence interval may be argued as being just over 1. However, the findings do suggest an association between focal GCC loss and diabetic neuropathy, as well as that between global GCC loss and age, independent of DR.

To recall, the ganglion cell complex comprises of inner plexiform layer, ganglion cell layer and nerve fibre layer. The inner retina is also occupied by other cells such as astroglial cells, amacrine cells, horizontal and bipolar cells (Zeimer et al., 1998). Therefore, loss in ganglion cell complex in the macular area may not be specific only to ganglion cells but can reflect loss in the above mentioned cells.

The conventional GCC thickness profile examined in earlier experiment did not show statistically significant relationship with diabetic peripheral neuropathy. Interestingly, there is significantly greater focal loss in GCC volume in relation to the severity of neuropathy. These findings are similar to that reported in previous studies wherein, pattern-based GCC parameters detected more abnormalities than the GCC thickness profile in glaucoma (Tan et al., 2009) and macular degeneration models (Garas et al., 2012).

Functional significance of loss in GCC volume

The scanned GCC area topographically corresponds to a 24-2 visual field plot of a Humphrey perimetry program. Figure 32 shows a Humphrey SITA Standard 24-2 visual field plot. The region within the dotted line topographically corresponds to a GCC scan area, extending 10˚ superiorly, inferiorly and nasally and 15˚ temporally.

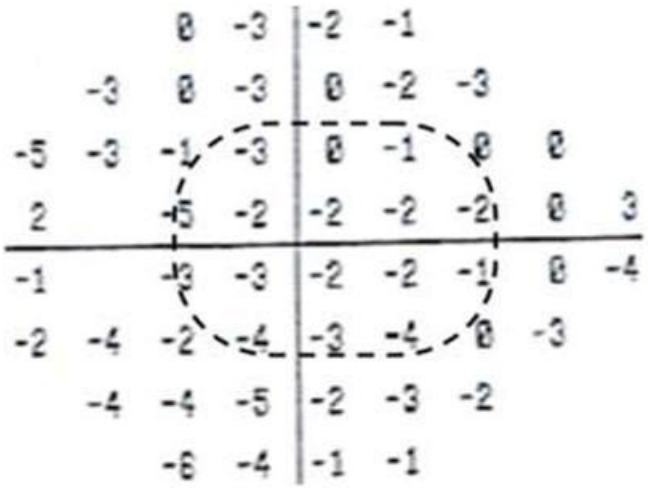


Figure 32. A visual field plot of a Humphrey 24-2 SITA standard program, adapted from Tan et al., 2009. Area inside the dotted line represents topographical correspondence with GCC area.

Therefore, loss of ganglion cells in the macular area may represent a significant threat to central visual field. Therefore, these findings warrant examination of central visual field.

To date, this is the first study to demonstrate pockets of inner retinal volume loss in relation to the severity of diabetic peripheral neuropathy; however, it is believed that these findings may pose a significant threat to the central visual field. In the first instance, initial studies may be indicated to examine the functional correlate in relation to loss in GCC volume in individuals with severe and less severe degrees of neuropathy.

5.7 Conclusion

The current research study demonstrated that individuals with diabetes have significant focal loss in GCC volume in relation to the severity of diabetic neuropathy, as well as a generalized GCC loss that is related to age. This loss is not significantly related to retinopathy, sex, duration of diabetes or the HbA_{1c} levels. The results from this study calls for examination of visual function in people with and without focal GCC volume loss in relation to diabetic neuropathy.

Chapter 6: Retinal tissue thickness in individuals who had undergone laser photocoagulation

6.1 Introduction

The participants who had undergone laser treatment for DR were excluded from the main experiment for the reasons that the effect of laser treatment can confound retinal thickness measurements. The purpose of this analysis was to examine if laser treatment had any effect on the retinal tissue thickness in the individuals who had undergone laser treatment for DR when compared with those with DR who did not have any laser treatment.

Laser photocoagulation is the standard treatment for severe NPDR and PDR (Neubauer et al., 2007). When laser is administered, the laser energy is absorbed by the RPE and photoreceptor layer and is converted to heat, resulting in coagulation or disruption to the RPE and photoreceptor layers. This in turn reduces the oxygen consumption and hence the demand. As a result, there is a decrease in the release of vascular endothelial growth factor, with a subsequent reduction in neovascularisation.

The clinical outcome is a prominent RPE burn. Although the target tissue is RPE, thermal and mechanical damage to other retinal layers has been observed in response to laser exposure (Marshall, 1970). For instance, early study in rabbits reported RPE being subjected to thermal damage from the laser, whereas, neural layers are subject to more of mechanical disturbances due to shifting of cellular components (Marshall., 1970). As an effect, the neural retina can sometimes be disturbed or damaged by heat conduction and can become oedematous and consequently lose its transparency. The temperature produced can be as high as 100˚ C leading to denaturing of proteins. The denatured proteins are then removed by phagocytosis and replaced by scar tissue (Berlien,

2003, p. 302) and this can have implications with regards to visual function (Marshall, 1970).

6.2 Types of laser treatment

Photocoagulation can be broadly classified into two (1) a scatter PRP, which involves a wide spread scatter (2) a focal laser for macular oedema.

Scatter laser photocoagulation also called **panretinal photocoagulation (PRP) is** a standard procedure that is recommended for severe NPDR and for PDR to reduce the risk of vision loss. Argon laser is used to create tiny burns in the peripheral RPE, thus carefully avoiding the macula, papillomacular bundle, and the optic nerve head. Once complete, it may take about 8 weeks for the new vessels to regress.

Focal laser photocoagulation is generally advised for diabetic macular oedema**,** after attempting optimal metabolic control. This can be either a focal treatment or a grid treatment.

Focal treatment refers to treating a microaneurysm (MA) directly in the presence of macular oedema occurring within 500-3000 μm from the centre of the macula. Individual MA's are treated with small spot sizes outside the foveal avascular zone (FAZ). The exudates disappear once the MA's are treated.

Grid treatment is primarily used for non-ischemic diffuse leakage without any focal or point leakage. The entire macula minus the fovea is treated with a low power grid laser to enhance the absorption of oedema. Grid laser is generally advised as a treatment to preserve the existing vision rather than improving the vision (American Diabetes Association, 1985).

Although recent techniques such as selective laser photocoagulation can be modified to produce the same effect on the retina with minimal damage to the

neighbouring tissues (Figueira et al., 2009), there is always some degree of damage.

6.3 Adverse effects

There is a risk of worsening of macular oedema, decrease in visual acuity and an increase in size of the lesion (Schatz H, 1991). Other side effects include inadvertent laser scarring at the fovea leading to central scotoma because of damage to photoreceptors (Framme et al., 2004), diminished night vision (Prskavec et al., 1986) and constriction of binocular peripheral visual fields (Pearson et al., 1998).

6.4 Retinal tissue thickness in laser photocoagulation

The RNFL thickness is reduced in eyes that have had laser when compared to that of eyes with DR without laser treatment (Cankaya et al., 2011; Hsu et al., 2002; Kim et al., 2009; Lim et al., 2009). In the study by Muqit et al (2009), the mean and the temporal quadrant RNFL thicknesses at 10 weeks post laser showed a significant increase when compared to baseline values. However, at 6 months, mean and temporal quadrant RNFL thicknesses reduced significantly to below baseline levels (Muqit et al., 2009). A recent study observed significantly increased RNFL thicknesses at 6 months after PRP but gradually decreased at 24 months below baseline values. In contrast, RNFL thickness in the temporal quadrant increased significantly at 6 months and reduced at 24 months but remained higher than the baseline readings. Central zone thickness showed a significant increase at 3 months and remained significantly higher at 24 months when compared to baseline readings (Lee et al., 2012). Another study showed an increase in RNFL thickness at 2 and 6 months after PRP but the difference did not reach statistical significance. However, the optic nerve rim area showed a significant increase from baseline to 2 months and at 6 months post PRP (Ritenour et al., 2009). The RNFL thickness was examined in the previous studies at various time intervals. However, collective evidence suggests that with longer time interval from the laser treatment, the RNFL showed a tendency

to decrease in thickness and appear to drop below baseline levels, despite any transient increase in thickness.

Optic nerve head photographs were examined in people with PDR just before and at one year after PRP; no difference was observed in the nerve head appearance (Johns et al., 1989). The visually evoked potential amplitudes did not show significant changes after PRP (Zhang et al., 2000). Photocoagulation was associated with an increase in the choroidal blood circulation (Takahashi et al., 2008) and a decrease in the retinal blood flow in eyes treated for PDR (Grunwald et al., 1989; Grunwald et al., 1986).

It appears that the RNFL shows intermittent increase in thickness but eventually reduces in thickness to below baseline levels after laser treatment for DR. The varying results reported in the above studies may be likely explained by differing clinical laser settings used in each study. The outcome depends on factors such as differences in absorption properties of lasers (Blankenship, 1986), laser settings such as the spot size, duration (length of pulse), power, wavelength of laser beam (Marshall, 1973), frequency of treatment (Shimura et al., 2003), focal length of the converging lens, absorption and the magnitude of the temperature increase in the tissue. This could be a likely explanation for the inconsistency in post-study changes.

6.5 Purpose

The purpose of this analysis was to explore the full retinal and inner retinal thickness in individuals who had undergone some form of laser treatment for DR, in comparison to individuals with DR who had not had laser treatment. The analysis was performed to determine if laser treatment significantly altered the retinal thickness. There were no criteria set for visual acuity or to any other parameter under consideration for either group.

6.6 Methods

The normality of distribution was tested. Clinical and neuropathy parameters were compared between individuals who had undergone laser treatment for DR

and that of people with DR using an independent samples t-test. There were differences (2 times) in sample size between the two groups; therefore a Levene's test for equality of variances was performed. In case of violation of Levene's test for equality of variances, a t-test not assuming homogeneous variances was utilised.

6.7 Results

There were 38 eyes of 38 participants that underwent laser treatment and 63 eyes of 63 participants with DR but had no laser treatment. For the purposes of this document, the groups are defined as 'Laser group' and 'DR group'.

Individuals in the laser group were on an average 6 years older (p = 0.005) and had 4 kg/m^2 lower BMI (p = 0.002); the vibration threshold was 7.8 Hz higher in individuals in the laser group. Other clinical parameters namely HbA_{1c} levels, duration of diabetes, total cholesterol, and blood pressure in the laser group were similar to that of DR group.

Other neuropathy measures such as the NDS, DNSS, warm and cold thresholds, warm and cold induced pain thresholds, peroneal nerve conduction velocity and amplitude and the number of monofilament points detected in the laser group were similar to that of DR group. Results are presented in Tables 31-32.

Table 31. Summary of clinical variables in the laser group compared to those in DR group

Variables	Laser group Mean ± SD n Min - Max	DR group Mean ± SD n Min - Max	*t*	df	*p*-value
Age	61.8 ± 9.3 38 43.1 - 76.8	55.8± 8.7 63 40.3 - 71.6	2.907	99	0.005
Duration of diabetes (years)	24.4 ± 15.4 38 6 - 55	24.9 ± 13.3 63 3 - 64	-0.204	99	0.839
BMI (kg/m^2)	27.2 ± 5.1 38 19.2 - 38.4	31.2 ± 6.1 63 22.5 - 55.5	-3.262	99	0.002
BP systolic (mmHg)	131 ± 20 38 101 - 201	129 ± 14 63 101 - 166	0.133	57	0.895
BP diastolic (mmHg)	74 ± 9 38 61 - 95	76 ± 8 63 58 - 97	-1.094	99	0.277
Total cholesterol (mmol/L)	4.6 ± 1.0 37 30. - 7.0	4.5 ± 1.1 63 2.6 - 8.6	0.401	98	0.689
QST warm sensation threshold (˚C)	41.7 ± 4.3 38 33.3 - 49.4	40.5 ± 4.6 63 32.8 - 50.0	1.588	99	0.115
Peroneal M amp ankle to EDB (millivolts)	2.5 ± 2.1 37 0.1 - 8.1	2.9 ± 2.6 63 0.1 - 14.1	-0.600	98	0.550

BMI, body mass index; BP, blood pressure; QST, quantitative sensory testing; amp, amplitude; EDB, extensor digitorum brevis

Table 32. Variables in the laser group compared to the DR group using non-parametric tests

Variables	Laser group Median IQR Min - Max	DR group Median IQR Min - Max	Mann-Whitney *U* test *p*-values
HbA_{1c} (%)	7.5 1 6 - 14	8.1 2 6 - 13	0.251
NDS	2 3 0 - 10	2 4 0 - 10	0.356
DNSS	1 2 0 - 4	0 2 0 - 4	0.061
Peroneal CV ankle to FH (meters/second)	41.8 9.6 20.0 - 50.0	41.7 7.5 20.0 - 56.6	0.498
QST cold sensation threshold (˚C)	23 10.1 0.0-31.2	26.5 8.2 0.0 - 31.3	0.077
QST cold induced pain threshold (˚C)	0.7 11.1 0.0 - 25.3	5.2 18.7 0.0 - 28.1	0.076
QST vibration threshold (Hz)	21.4 34.9 1.4 - 128.4	13.6 20.4 2.1 - 130.0	0.023
Monofilament no. of points detected out of 3	3 1 0 - 3	3 1 0 - 3	0.757

IQR, inter-quartile range; NDS, neuropathy disability score; DNSS, diabetic neuropathy symptom score; CV, conduction velocity; FH, fossa head; QST, quantitative sensory testing

Retinal thickness in the laser group and the DR group

The full retinal and inner retinal thickness in the laser group did not differ significantly from that of the DR group. Results are presented in Table 33.

Table 33. Retinal thickness in the laser group compared to that of DR group

Retinal tissue thickness (μm)	Laser group Mean ± SD Min - Max	DR group Mean ± SD Min - Max	*t*	df	*p*-value
Thickness in the central 1mm zone	248 ± 28 183 - 309	243 ± 25 140 - 293	0.512	96	0.610
Overall parafovea	303 ± 26 218 - 339	304 ± 17 259 - 337	-0.264	54	0.793
Overall perifovea	266 ± 19 229 - 307	267 ± 16 201 - 301	-0.220	96	0.826
Overall RNFL	97 ± 12 65 - 117	102 ± 12 71 - 132	-1.402	97	0.164
Overall GCC	94 ± 12 65 - 119	95 ± 8 77 - 117	0.075	53	0.941

RNFL, retinal nerve fibre layer; GCC, ganglion cell complex

6.8 Discussion

This analysis examined the full retinal and inner retinal thickness in individuals who had undergone laser in comparison to those with DR but have not had laser treatment. Initial analysis of clinical parameters showed that individuals in the laser group were significantly older and also had significantly lower BMI, and a higher vibration threshold than those in the DR group. The laser group differed from the DR group in terms of DNSS, cold sensation threshold and cold-induced pain threshold but the differences did not reach statistical significance. It is interesting to note that the full retinal and the inner retinal thickness in the laser group did not differ significantly compared to the DR group. However, the results are not surprising because several factors (described below) may have influenced the outcome.

For instance,

a) Clinical laser settings such as spot size, pulse duration, frequency of shots per sitting, number of sittings was not known. These settings can influence the extent of thermal damage at the level of retinal layers. This could have introduced some variability in the results as reported in literature.

b) The stage of retinopathy before laser was not known. In general, scatter laser treatment is advised for severe NPDR and for PDR and a focal laser for macular oedema. In the current study, most participants in the DR group had individuals with very mild NPDR or mild NPDR. It is likely that the lack of difference between the two groups may be explained by these differences. For instance, it is possible that the retinal layers may be oedematous in the presence of retinopathy or may be thinner due to scar formation. Firstly, as demonstrated so far from the current project, the retinal layers are reduced in relation to neuropathy. However, in this part of the study, neuropathy measures in the laser group were not significantly different compared to the DR group, thus leaving a greater possibility of retinal tissue thickness being influenced by retinopathy

more than neuropathy in the laser group. Therefore, it is likely that the increased retinal thickness may be associated with higher degrees of DR as shown in previous literature (Chapter 2). Secondly, it is also likely that this effect may have been cancelled out by the thinning of the RNFL after laser.

c) The laser group comprised of participants that had undergone focal laser or scatter laser. Both the groups were combined for this analysis. This may have attributed to some variations within the laser group and as a result, showed no significant differences between the two groups.

d) OCT was not performed at pre-determined time points. As a result, the time interval between the date of laser and the date of OCT examination varied between participants. This could introduce variability in the result as reported in literature wherein, the RNFL shows intermittent increase in thickness but eventually reduces in thickness to below baseline levels. For the reason that OCT was not performed at fixed time points post-laser, it is likely that this laser group have had some individuals with transient increase in RNFL thickness and some individuals with thinner RNFL and as a result, the combined effects may have cancelled each other. However, these are assumptions only.

Therefore, the question whether laser treatment is a confounding factor for the retinal thickness measurements, is still unclear. Analysis of retinal tissue thickness in the same group before and after laser may contribute to more relevant information and may provide a more sound appreciation of the effect of laser on the retinal tissue thickness. However, this is not the objective of the current research program.

Nevertheless, the observation that the individuals in the laser group had significantly higher vibration threshold is noteworthy because the individuals may be at risk of impending foot ulceration and hence require follow-up care.

6.9 Conclusion

This supplementary analysis showed that the retinal tissue thickness in participants who had undergone laser treatment was not significantly different from those in the DR group. However, individuals in the laser group had significantly higher vibration threshold, which suggests that they may be at risk of foot ulceration. Shahidi et al (2012) observed that the inferior RNFL thickness is thinner in those at risk of foot ulceration. In the current study, although the retinal tissue thickness did not vary at the moment, these individuals need follow-up care to monitor especially the inner retinal thickness and the visual function.

Chapter 7: Summary, overall significance of this research

This research program has demonstrated that retinal structural integrity as indicated by full retinal thickness, macular and the peripapillary inner retinal thickness is compromised in relation to diabetic peripheral neuropathy. This research program has investigated several important research questions which are addressed in this chapter.

There is uncertainty as to whether the type of diabetes influences retinal tissue thickness. To understand this, the following research question was addressed.

1. *Is there is a relationship between the type of diabetes and retinal tissue thickness?*

No. The type of diabetes as well as the interactions between type of diabetes and age, sex, diabetic retinopathy, neuropathy disability score, duration of diabetes and HbA_{1c} levels are not significantly related to the retinal tissue thickness. However, individuals with type 2 diabetes have a tendency for reduced retinal thicknesses compared to those with type 1 diabetes; therefore, individuals with type 2 diabetes may need close monitoring of the retinal tissue thickness integrity as well as examination of the associated visual function. Also, examination of other risk factors such as cardiovascular or nephropathy-related factors may need evaluation in order to understand the reasoning behind reduced retinal thickness in type 2 diabetes.

2. *Are the full retinal thickness and inner retinal thickness significantly related to the severity of diabetic neuropathy, defined using NDS criteria?*

Yes. Both the full retinal thickness and inner retinal thickness are reduced in individuals with advanced degrees of neuropathy. For instance, the retinal thickness in the perifovea is decreased in relation to diabetic peripheral

neuropathy. This may reflect a compromise in the photoreceptor layer. Furthermore, the thickness in the parafovea is reduced in the presence of DR. The RNFL is significantly thinner in individuals with advanced degrees of neuropathy. The GCC thickness showed a tendency to be reduced in relation to diabetic peripheral neuropathy. These findings may be reflected as compromised central and peripheral visual field sensitivity.

3. *Are the focal and global losses in GCC volume significantly related to diabetic peripheral neuropathy?*

Yes. In individuals with diabetes, there is significant focal loss in GCC volume that is related to the severity of neuropathy. There is a significant global loss in GCC volume associated with age. This may be mirrored as a reduction in visual field sensitivity in the central field or a compromise to the visual function and therefore warrants investigation.

DR has been proposed as a putative mechanism behind neural apoptosis, loss of ganglion cell bodies and glial tissue in diabetes until recently where reduced retinal nerve fibre layer thickness has been reported to be related to diabetic peripheral neuropathy rather than retinopathy (Shahidi et al., 2012). This opened up the possibility that the ganglion cells and the other retinal layers may be involved in relation to diabetic peripheral neuropathy. In addition to reduced retinal nerve fibre layer thickness, this research program has demonstrated that there are focal losses in ganglion cells that are related to DPN rather than DR; these may indicate changes to the axon, axonal diameter, glial structure, extracellular volume, retinal vessel diameter or a combination of the above. In fact, this further substantiates the hypothesis that retinal neuropathy in diabetes may be related to systemic neural injury, as a result of microvascular changes or that from chronic hyperglycaemia and may invovle multiple layers of the retina. Furthermore, the involvement of other cranial nerves suggests a multi-system involvement affecting all branches of the nervous system in diabetes. For example, extra ocular muscle palsies arising from damage to III, IV and VI cranial nerves, depletion of retinal nerve axons that form a part of II cranial nerve, alterations to corneal nerves that constitute the V cranial nerve, pupillary

defects (may indicate autonomic dysfunction) and so on. The findings from the current research program have important implications for the potential compromise to the central and peripheral visual field sensitivity. Therefore examination of visual fields in individuals with diabetes with neuropathy may be indicated.

7.1 Limitations, overall strengths of this research program

Visual function was not assessed as a part of this study. Therefore, the clinical significance of the observed retinal structural compromise is not well understood. This may be viewed as a limitation in this study.

The strength of association between retinal structural thinning and diabetic neuropathy was not high, which could be regarded as a limitation in this study. The highest R^2 observed was 19% for inferior RNFL thickness, indicating that one-fourth of the reduction in the RNFL thickness is related to the severity of diabetic neuropathy and age. This suggests that there are other variables yet to be explored, that may influence the retinal tissue thickness to a greater extent. A likely explanation for the weaker strength of association could be that, the study was started with a pre-defined research objective: 'to determine the relationship between the retinal tissue thickness and diabetic peripheral neuropathy'. The association was determined while adjusting for the key variables namely DR, age, gender, duration of diabetes and the HbA_{1c} levels. The rationale for choosing these variables was that these are consistently reported as risk factors for both DPN and DR and are related to the retinal tissue thickness as well. Therefore, the results obtained are within the bounds of the research objective, taking into account, the pre-defined set of key variables. However, an exploratory analysis (model building) could have revealed additional variables that influence the retinal thickness. The method would involve analysing the retinal thicknesses and adding or removing variables that would significantly contribute to the overall statistical model by improving the adjusted R^2. However, the sample size will also need to be higher to be able to include many independent variables into the model. A ratio of 20:1 may be considered as a guideline for the sample size needed to account for the number

of independent variables to be entered into the regression models. That way, the other micro- and macrovascular factors may also be studied. This exploratory analysis may lead to a better model with few other variables that might significantly influence the retinal tissue thickness. The current study can be considered as a foundation upon which, future studies can be developed, taking into account, the other cardiovascular and nephropathy related factors as well.

In the current project, diabetic retinopathy (DR) did not show a significant relationship to the inner retinal thickness. It is pertinent to mention that DR here represents 'clinically visible signs' only. There may also be subtle retinopathy changes that may be subclinical; therefore, the absence of clinical signs of DR does not necessarily rule out DR. Therefore, the results must be interpreted with caution.

In addition, duration of diabetes was by self-report. The exact day of diagnosis cannot be firmly determined. Therefore, this fact needs to be taken into account.

Despite any short comings, this research program has answered novel research questions within the constraints of the key variables assessed, and has contributed to new knowledge regarding the retinal anatomy in relation to diabetic peripheral neuropathy. These findings have important implications to visual function, mobility and quality of life issues.

Clinical significance of this research and contribution to new knowledge

This is the first study to examine the association between the type of diabetes and retinal tissue thickness while adjusting for certain important confounding variables. To date, this study is the first to demonstrate compromised neural layer thickness in the macular and peripapillary regions, as well as reduced full retinal thicknesses, in relation to diabetic neuropathy. This research program has contributed to a deeper understanding of the changes to the retinal anatomy in relation to diabetic peripheral neuropathy. These anatomical changes may be mirrored as compromise to central and peripheral visual fields. This remains to be tested.

This is the first study to demonstrate pattern-based ganglion cell loss in relation to diabetic peripheral neuropathy that is not related to DR. This is a new observation but is also a critical finding in that, a compromise in the macular region can represent a significant threat to central visual field. Therefore, to understand the clinical implications of these structural deficits, examination of the central and peripheral visual fields, as well as assessment of ganglion cell function may be indicated. In addition, the observation that the perifoveal thickness is reduced may reflect changes to the photoreceptor layer. Therefore, this necessitates evaluation of the photoreceptors. The following sections provide a review of visual function tests that may be helpful in the assessment of structural compromise in diabetic neuropathy.

7.2 Examination of the visual function

Visual function is a broad terminology that includes measures such as visual acuity, contrast sensitivity function, hue discrimination, dark adaptation and visual fields (Nasralah et al., 2013). The following sections discuss the various attributes of visual function.

It is of interest to note that foveal anatomy is suited for highest discriminative ability and colour perception. Assessment of visual acuity and colour vision may therefore be valuable.

Visual acuity

Visual acuity (VA) is a measure of spatial resolution of the retina. Visual acuity indicates resolving power of the eye, (Hartridge, 1922) and is typically assessed by visual recognition of letters on a chart. Visual acuity has been utilized as a measure of visual function to monitor and to record progression of disease in clinical trials, as well as to follow-up after treatment. However, in diseases like DR, VA deterioration is frequently observed in late stages of disease such as proliferative DR or in the presence of macular oedema (Antonetti et al., 2012). This suggests that visual acuity may not be the best measure of visual function especially in early stages of the disease. There are other measures of visual function that appear to be sensitive to subtle retinal changes such as colour

vision, contrast sensitivity, perimetry and electrophysiology (Jackson et al., 2012).

Colour vision

Tritan (blue-yellow) colour vision defects have been observed in individuals with diabetes even in the absence of visible signs of DR (Ong, et al., 2003). When compared to those without diabetes, the error in colour discrimination was significantly higher in those with diabetes without DR (Green, et al., 1985) (Hardy, et al., 1992). Acquired macular diseases tend to be associated with blue-yellow defects, whereas optic nerve lesions can produce red-green defects (Wright et al., 2007). The current project has demonstrated the involvement of both macular and peripapillary neuroretinal layers in relation to diabetic peripheral neuropathy. It would therefore be interesting to examine the colour vision defects in relation to diabetic peripheral neuropathy.

The FM-100 hue test is a commonly used test that is sensitive for detecting defects in colour discrimination. The test consists of four boxes of coloured caps with fixed caps at either ends; the task for the individual is to arrange the caps as a gradual progression of the colours. The bottom of the caps has numbers printed for reference to indicate the correct sequence. The total number of deviations in the sequence is recorded as the total error score (Farnsworth et al., 1957). In the current research project, the reduction in perifoveal thickness indicates that the outer retinal layers may be involved. It is therefore likely that the photoreceptors are involved and this may manifest as blue-yellow or red-green colour vision defect; this remains to be tested. The FM-100 hue test may be utilized to assess in-detail the nature of colour vision defects if any, in relation to diabetic peripheral neuropathy.

Implications of defective colour vision in everyday life

Problems in colour vision may pose challenges in everyday life. For instance, while cooking, individuals may have difficulty in determining if the meat is adequately cooked based on the colour; difficulties can rise in choosing

wallpapers and cosmetics (Steward et al., 1989). Difficulty may arise while choosing fruits for their ripeness based on their colour; for instance, banana.

Children are not spared when it comes to diabetic neuropathy (Blankenburg et al., 2012). Children are generally associated with a world of colours. Colour vision problems especially in children can be more problematic to deal with. Defective colour vision can make their food appear less appealing. As a result, they may dislike those foods that appear less colourful. There can also be issues at school where there are creative projects involved such as drawing or crafty work (Neitz et al., 2000).

Issues may arise in selecting clothing. Someone with defective colour vision may choose very bright or vibrant colours as they are not aware that they are brighter than normal. Defective colour vision can interfere with occupational duties related to transportation, the food industry, textile industry and armed forces where, for example, interference with the interpretation of emergency flare, indicator lights can be problematic. Earlier, it was thought that individuals with defective colour vision may not be able to drive vehicles because they may have difficulty following the traffic lights; however, individuals can still use other cues (Cole, 2004) such as the order of the light on a signal. However, this is not always the case. For instance, the lights can have arrows that change colours or may be arranged from left to right as in selected cities in the US. These can be challenging for a new driver or driver in a new zone. Therefore, assessing colour vision may be valuable so that individuals can be counselled accordingly.

Photostress test

The photostress test can differentiate macular involvement from an optic nerve involvement. The test involves exposing the macula to a bright source of light, like an ophthalmoscope or a penlight for about 10 seconds. This would bleach a significant proportion of visual pigments. The test is performed monocularly. After exposure to light, the time taken for the eye to read within a line from the previously read line is recorded. The response is considered normal if read

within 50-60 seconds. The rationale is that the return of normal retinal function and sensitivity depends on the regeneration of visual pigments. Disease affecting photoreceptors, Bruch's membrane, choriocapillaries or choroid can prolong the visual recovery time. No such prolongation is seen in optic nerve conduction defects (Margrain et al., 2002). This test may show abnormality if the photoreceptors are involved in relation to reduced perifoveal thickness. Nevertheless, the test is not useful to quantify the extent of macular involvement.

Contrast sensitivity

Contrast sensitivity (CS) is defined as the ability of the visual system to distinguish an object from its background. If the visual acuity is reduced, the contrast sensitivity is reduced as well. On the contrary, CS may be reduced irrespective of the visual acuity. Therefore, if visual acuity alone is tested, the relative impairment in CS will be undefined.

Contrast sensitivity may be affected early in a disease process. For instance, in the absence of clinical signs of DR, impaired contrast sensitivity has been reported in diabetic children (Georgakopoulos, et al., 2011) as well as in diabetic adults (Heravian, et al., 2010; HyvÄRinen, et al., 1983; Trick, et al., 1988) while visual acuity was unaffected. A trend of further reduction in CS has been observed in individuals with early to advanced stages of DR (Ismail, et al., 1998). The explanation for the impaired contrast sensitivity in diabetes is multi-fold; one of them being hyperglycaemia. Although no direct cause-effect relationship could be established between hyperglycaemia and CS, impaired CS was found to be associated with increased retinal blood flow during alterations in blood glucose levels with glucose clamp technique (Bursell, et al., 1996), thus indicating an indirect link between the higher glycaemic levels and CS. Another likely explanation could be alterations in retinal and choroidal blood supply in diabetes that have been linked with impaired CS (Shoshani, et al., 2011). Loss in CS has been associated with capillary dropout in individuals with diabetes (Fletcher, et al., 2005). The above studies indicate that the contrast sensitivity function may be a sensitive tool for determining loss in visual function.

Contrast sensitivity may be assessed using a grating or an optotype (Mäntyjärvi et al., 2001). Grating indicates the spatial frequency or the number of dark-light cycles per visual angle. In early stages of macular diseases, there is marked impairment of CS function at intermediate and higher spatial frequencies (Sjostrand et al., 1977) and dramatically reduced CS across a wide range of frequencies in advanced stages of macular diseases.

Contrast sensitivity chart such as Pelli-Robson chart may be also beneficial. The chart consists of 8 rows of letters of 20/60 equivalent that vary in their contrast from left to right. The set of letters with lowest contrast identified by the participant is recorded. A score of 2.0 indicates normal contrast sensitivity, while a score less than 1.5 indicates impaired contrast sensitivity function (Pelli et al., 1988). A combination of both optotype and grating may be utilized for contrast sensitivity testing.

Implications of abnormal contrast sensitivity

Abnormal CS can make objects appear foggy or washed out. Impaired contrast sensitivity can lead to problems in judging distances, climbing stairs, and recognising faces (Nema et al., 2010). Patients may bump into objects or may be prone to falls and fractures (de Boer et al., 2005). Patients with diminished CS can have driving-related problems despite normal visual acuity (Puell et al., 2004). Driving can be difficult in low lighting conditions (Szlyk et al., 2005) such as during dawn, dusk, overcast days, rain and fog (Woods et al., 1995). Older drivers experience decreased ability to drive at night. Drivers with diminished contrast sensitivity function and a greater sensitivity to glare avoid night driving (Wood et al., 2002). Contrast sensitivity testing can therefore be beneficial so that appropriate measures can be taken to counsel and to educate the individuals about tasks involving contrast sensitivity. Yellow filters may be tried as a treatment option for improving the contrast (Lee et al., 2002).

Visual fields

Mid-peripheral visual field sensitivity is reduced in relation to diabetic peripheral neuropathy, and this was not explained by DR (Sampson et al., 2012).

Previous studies have demonstrated neuroretinal degeneration even in the absence of clinical signs of DR (detailed in Chapter 2). The results from the current study demonstrate that the compromise in neuroretinal layers is related to diabetic peripheral neuropathy. This emphasizes the importance of visual field assessment as part of routine eye examinations even in the absence of clinical signs of DR.

Perimetry is a measurement of sensitivity to light across the retina. With manual kinetic perimetry, scotomas in the visual fields were detected in a group of individuals with and without DR (Roth, 1969). In the DR group, the scotomas topographically corresponded to retinopathy lesions. However, in the no DR group, the presence of scotomas was considered as a pre-retinopathy sign.

In a study using static perimerty also called standard automated perimetry (SAP), about 4% of the eyes with very mild and mild DR had mean deviation (MD) values (overall depression or sinking of the visual field) with a probability value worse than 5% (Henricsson, et al., 1994), suggesting that a vast majority of subjects with and without DR had MD within normal limits. However, the number of abnormal test points became higher with greater severity of DR. A similar trend was observed by Park et al (2011) where the mean deviation values increased with the severity of DR.

A limitation with the use of white-on-white perimetry is that there is already 30-40% loss in retinal nerve fibre axons before any obvious field defect appears (Quigley, et al., 1982). However, blue-on-yellow perimetry (blue target on a yellow background) has been found to be superior in detecting depressions in visual field, when white-on-white perimetry results were normal (Nitta, et al., 2006). Nevertheless, the reduced sensitivities on blue-on-yellow perimetry could also be due to yellowing of the crystalline lens. Interestingly, with the use of red-on-white perimetry, significantly greater visual field defects were demonstrated and because of the longer wavelength, it is not affected by cataract or yellowing of the lens (Zele, et al., 2008).

With the use of alternate visual field techniques, such as flicker perimetry, researchers have successfully demonstrated reduction in visual field sensitivity within 6˚ from fixation, in individuals without DR and also in those with early stages of DR (Stavrou, et al., 2005). These defects were not detected by the static white-on-white perimetry. Flicker perimetry can be performed using critical fusion frequency (CFF) or temporal modulated perimetry (TMP) techniques (Stavrou & Wood, 2005). The CFF involves measuring the threshold frequency by presenting alternating or flickering dark and light bars at a fixed level of contrast. The highest temporal frequency detected is recorded as the threshold. The TMP involves measuring the threshold at varying levels of contrast that flicker at a constant rate. The stimulus with lowest contrast detected is recorded as the threshold. The rationale is that identification of flicker requires greater metabolic demand and therefore can show greater abnormality in the presence of vascular compromise (Scheiner, et al., 1994). In addition, flicker sensitivity also depends on the integrity of photoreceptor and their metabolism. When compared to the two techniques, the TMP technique is reported to be more effective than the CFF in separating glaucoma patients from normal non-glaucomatous healthy individuals (Yoshiyama et al., 1997).

Other perimetry techniques namely frequency doubling perimetry and microperimetry has been in use in clinical settings.

Frequency doubling perimetry

Frequency doubling perimetry (FDP) was initially developed based on the hypothesis that when a low spatial frequency grating is allowed to undergo counterphase flicker, the spatial frequency appears to be doubled (Kelly, 1966; Zeppieri et al., 2008). It was hypothesized that this illusion is a property of a subset of ganglion cells that is involved in detection of movement and flicker (Derrington et al., 1984). The FDP was reported to be superior to standard automated perimetry (SAP) in detecting early neuronal loss in glaucoma disease models. Also participants with diabetes had higher test scores and took longer testing time compared to those without diabetes, when SAP results were normal, suggesting neural degeneration in diabetes. However, impaired contrast

sensitivity can confound the FDP results independent of any neuronal loss (Realini, et al., 2004).

Interestingly, recent study has reported that a Goldman size III stimulus may be more effective than frequency doubling technology in testing a specific subtype of ganglion cells (Swanson et al., 2011). However, the threshold measurements from the standard automated perimetry represent responses of multiple levels of the visual pathway.

Microperimetry

Microperimetry is advancement in perimetry technique with which, clinicians are able to map the retinal sensitivity with a real time topographical correspondence with the retina. Microperimetry detected greater number of individuals with visual field defects compared to that with standard automated perimetry (Lima et al., 2010).

There are two types of technology available in microperimetry. One technology uses digital fundus photography and computerized perimetry; the other uses scanning laser ophthalmoscope to combine SD-OCT with microperimetry for the central 20˚ of the retinal field of view. A dotted light is projected directly onto the retina through the pupil at certain intensity at predefined points on the retina. The patient is asked about the perception of the light at certain brightness. By changing the light intensity, the differential light sensitivity or threshold at the retinal points can be determined. When compared to the conventional perimetry, microperimetry provides a real time ophthalmoscopic imaging and point to point topographical correlation of functional deficits with the fundus pathology (Sunness et al., 1995) and can detect areas of reduced retinal sensitivity in the presence of normal standard automated perimetry (Lima et al., 2010) in individuals with glaucoma.

Implications of compromised visual field sensitivity

Loss in visual field sensitivity can result from neuroretinal compromise (Quigley et al., 1982) as well as from damage or death of the photoreceptors (Milam et al., 1998).

A GCC scan corresponds to a Humphrey 10-2 visual field program. Loss in the ganglion cell complex in this region may be mirrored as loss in the very central portion of the visual field. This can present as difficulty in reading, as the central 10˚ of the visual field is crucial for reading and is represented by 50% of the primary visual cortex (Demb et al., 1997). Visual field sensitivity loss can lead to disturbances in mobility and problems in climbing stairs can lead to falls (Freeman et al., 2007); difficulty in recognizing road signs (Wood et al., 2010), changing lanes, and manoeuvring (Johnson et al., 1983) and also difficulty in tracking moving objects (Owsley et al., 1995) may be observed. Constriction of useful visual field is associated with an increased risk of crash among elderly drivers (Owsley et al., 1999). Involvement of RNFL, GCC and photoreceptors can be a source of scotomas in the visual field as well as impaired dark-light adaptation (Framme et al., 2004).

Limitations of perimetry techniques

Perimetry techniques do have certain limitations. Collective evidence from glaucoma disease models have demonstrated that the structure versus function correspondence can depend upon a number of factors (Wood et al., 2000). The responses may vary due to discrepancy between the type or the extent of ganglion cell involvement and the nature of visual field testing system utilized (for example static versus flicker perimetry or white-on-white perimetry versus blue-on-white perimetry and so on).

Furthermore, there is a possibility that the structural damage may occur much earlier than a demonstrable functional loss, as in glaucoma disease models. For instance, nearly 70% loss in the ganglion cells in the parafoveal retina is required to demonstrate a 3-5 dB loss in visual field sensitivity (Harwerth et al.,

1999; Harwerth et al., 2004). As a result, perimetry techniques may not be sensitive to detect early losses in ganglion cells.

Various other techniques such as electrodiagnostics are available that can be utilized to objectively measure the visual function and they also exhibit topographical correspondence with retinal regions examined.

Electrodiagnostic tests

Electroretinogram (ERG)

Full-field ERG represents the mass electrical response from the entire retina that constitutes rod and cone-mediated responses. Studies that measured the retinal electrophysiological responses in individuals with diabetes observed reduced oscillatory potential amplitudes and delayed latencies (Barber, 2003) in the absence of signs of DR; this suggests susceptibility of the middle and inner retinal layers (Kern et al., 2008) and possibly amacrine cells to vascular insult. However, unless about 20% of the retina is affected, the full-field ERG responses are generally normal (Hood et al., 2008). Therefore, full-field ERG may not be suited for assessing focal retinal function or macular function.

The multifocal ERG (mfERG) on the other hand, provides a topographical measure of the retinal function from the central 45 ° of the retina (Hood et al., 2012). The responses are essentially cone-driven, elicited from the foveal, macular and paramacular regions. The outcomes of mfERG are a result of mathematical extraction and are recorded as a waveform represented as 5 rings. The waveform has a first negative deflection called N1, followed by a first positive P1 and then a second negative N2. Similar to a full-field ERG, the N1, P1 and N2 represent responses from photoreceptors, bipolar and amacrine cells as in full-field ERG. Interpretation involves assessment of amplitudes and implicit times in comparison to age-related normal values. The mfERG amplitude is measured from trough to peak and the implicit time is measured as the time at which P1 occurs. The advantage of mfERG over full-field ERG is that the responses can be localized to separate retinal regions such that, loss of signal can be localized to dysfunction in that region. A reduction in the mfERG

amplitudes with delayed implicit times may suggest ischaemic or hypoxic effects; however, the technique requires central fixation. More importantly, the retinal ganglion cells have little contribution to the mfERG responses (Hood, 2000).

Another variation of ERG technique is the pattern ERG (PERG). Pattern ERG responses represent a measure of ganglion cell function (Holder et al., 2007) from a 15 ˚ field (Neveu et al., 2006). The retinal response is obtained by having the patient view a checker board pattern stimulus that is based on temporal modulation. Reduced amplitude and or a delay in the implicit time may represent disease process affecting the outer plexiform layer and bipolar cells that connect with the ganglion cells. The PERG responses were affected in individuals with diabetes with and without DR (Coupland, 1987; Falsini et al., 1989; Fortune et al., 1999; Ghirlanda et al., 1991; Prager et al., 1990) thus supporting the hypothesis that neural degeneration may be possibly related to diabetic peripheral neuropathy. This indicates that PREG can detect early neuroretinal changes in diabetes. Therefore, PERG may prove to be beneficial in demonstrating peripheral neuropathy related compromise in ganglion cell function, irrespective of DR.

Visual evoked potentials (VEP)

Visual evoked potentials represent electrophysiological responses recorded from the visual cortex, by attachment of electrodes to the scalp. The VEP represent an objective method of assessing optic nerve function. Flash VEP and pattern VEP are the two commonly performed VEP techniques. Flash VEP is performed to assess the visual function wherein, visual acuity is not a main requirement for the conduct of the test. Pattern-reversal VEP on the other hand requires the participant to view checkerboard stimuli that alternates. Pattern VEP responses depend on central fixation and hence visual acuity. The waveform has the first negative deflection, the N75, followed by the P100; P100 is the first positive rise on the response waveform. This is followed by N135 which is the second negative. The latency and the amplitude of P100 are mostly of interest. The latency of this P100 has been demonstrated to be delayed in

people with diabetes in the absence of clinical signs of DR, which normalized following optimal metabolic control (Verrotti, et al., 2000). The amplitude is generally contributed by the axonal numbers, and the latency is an attribute of the myelin status of the entire visual pathway (Daube. et al., 2009). About 65% of the response from pattern VEP represents that from the central 2 degrees of fixation indicating that it is not particularly useful for measuring peripheral retinal function. In addition, pattern VEP is subject to high variability between subjects (Baseler et al., 1994) and is limited by the reduced ability to localize the defects in space (Danesh et al., 2006).

The multifocal VEP (mfVEP) involves objective evaluation of retina, optic nerve function and visual cortex; tests up to 26 degree eccentricity (Klistorner et al., 1998); has better repeatability compared to that of standard automated perimetry (Fortune et al., 2006). However, the responses can be confounded by background noise due to the morphology of the visual cortex (Hoffmann, 2008); mfVEP also has been reported to have greater variability with increasing eccentricity as well as a greater inter-test variability (Klistorner et al., 2005).

Assessment of visual function in diabetic neuropathy

Individuals with neuropathy and foot complications may have gait problems. They may bump into objects because of unsteadiness while walking. This may become more problematic especially in dim illumination. Therefore evaluation of contrast sensitivity may reveal useful information.

In the current study, a significant reduction in the full retinal thickness and neural layer thickness in the macular area and focal loss in ganglion cells is observed in individuals with diabetic peripheral neuropathy. Macular integrity is crucial as it subserves central visual field. Therefore, objective evaluation of both central and peripheral visual field sensitivity is indicated for such patients. Although microperimetry is a subjective technique, it represents an innovative technology among the perimetry techniques in that, it provides a topographical correspondence with the OCT scanned area as well as with ocular fundus images; therefore, the SD-OCT combined with microperimetry may prove to be

beneficial in this scenario. A pattern ERG may be utilized for assessment of ganglion cell function. The observation that the thickness in the perifovea is reduced could mean that the photoreceptors are involved; the results therefore call for examination of colour vision.

From the above tests, a comprehensive evaluation that includes colour vision test, contrast sensitivity evaluation, SD-OCT combined with microperimetry, and pattern ERG evaluation, may prove to be beneficial in mapping the visual function in individuals with diabetes with and without neuropathy. New technologies are developed in an effort to overcome the limitations of techniques, but essentially all tests of visual function are subject to certain limitations. Eye care professionals to choose the most appropriate test depending on the objective of the testing.

7.3 A potential marker for diabetic peripheral neuropathy

The term 'biomarker' has been defined by Hulka and colleagues (1990) as "cellular, biochemical or molecular alternations that are measurable in biological media such as human tissues, cells or fluids" (Hulka, 1991); for example, glucose levels in diabetes and blood pressure in hypertension. The main characteristic of a biomarker is that it should be specifically associated with a particular disease or disease state; must be capable of predicting the occurrence of the disease and be able to differentiate between similar physiological conditions. The Food and Drug Administration recommends that an ideal marker must be rapid, simple, accurate and an inexpensive measure.

The biomarker identification process may involve direct or indirect measurements of the proposed parameter. Direct sampling includes examination of the biological fluids such as blood or tears. Indirect sampling involves detection of changes to biological tissues by imaging techniques (Mayeux, 2004). Markers that represent a characteristic or feature identified in an image that is relevant to the diagnosis of the patient are called imaging markers (Thrall, 2003). Corneal nerve fibre pathology is an example of a marker where the images can be used to assess diabetic peripheral neuropathy

(Edwards et al., 2012; Edwards et al., 2012; Efron et al., 2010; Pritchard et al., 2010; Pritchard et al., 2011; Pritchard et al., 2012). Biomarkers can also be diagnostic or predictive (Jain, 2010).

Markers for diabetic peripheral neuropathy

There have been several breakthroughs in biomarkers research in various fields of medicine. For instance, samples of tissues such as the nerves, skin and muscles have been examined to understand the nature of the neurological disease process, as well as for developing effective treatment strategies.

Sural nerve biopsy and skin biopsy are the traditional tests for detecting small fibre neuropathy (Tavee et al., 2009). However, the biopsy technique is minimally invasive and therefore uncomfortable for the individual (Polydefkis et al., 2001); also the results cannot be obtained immediately. Magnetic resonance imaging of the spine can reveal nerve damage (Thakkar et al., 2012) but this technique is not cost-effective and is also not feasible in a clinical scenario. Other methods such as nerve conduction studies provide with an objective evaluation of nerve fibres but of predominantly large nerve fibres. Techniques such as quantitative sensory testing involve evaluation of both large and small nerve fibres (Vinik et al., 1995); however, this is a subjective test and also requires the use of sophisticated equipment. In addition, these techniques could not demonstrate nerve regeneration in the peripheral limbs (Hsieh, 2010). The eye serves as a suitable platform wherein, the nerve fibre involvement can be assessed non-invasively, with instant results.

Corneal nerve fibre morphology evaluated by corneal confocal microscopy (CCM), has been identified as an ophthalmic biomarker for diabetic peripheral neuropathy. The nerve fibre density in both the epidermis and cornea were significantly reduced in individuals with neuropathy compared with those without neuropathy (Quattrini et al., 2007). The CCM can differentiate between various degrees of neuropathy (Tavakoli et al., 2011) and can detect corneal nerve fibre regeneration after pancreatic transplantation (Mehra, et al., 2007) thus demonstrating that CCM is a potential surrogate marker for diabetic

peripheral neuropathy (Malik, et al., 2003). In addition, corneal sensitivity evaluated by means of non-contact corneal aesthesiometry is a useful marker and a threshold of 0.66 millibars or greater indicates the presence of neuropathy (Pritchard, et al., 2012; Pritchard, et al., 2010). Thus CCM has been proven to be successful in identification of diabetic neuropathy changes as reduced corneal nerve fibre length even in early stages of the disease (Edwards et al., 2012; Edwards et al., 2012; Efron et al., 2010), thus indicating that this may have the potential to serve as a quick screening tool.

Retinal markers for diabetic peripheral neuropathy are currently under investigation. The inferior RNFL thickness is compromised in individuals with neuropathy especially in those at risk of foot ulceration (Shahidi et al., 2012). Further to this, the current project has demonstrated that the full retinal thickness, macular and peripapillary nerve fibre layer thickness is compromised in relation to diabetic peripheral neuropathy. There is a greater focal loss in ganglion cell volume in individuals with neuropathy and it is worse in individuals with advanced degrees of neuropathy. Interestingly, focal loss in GCC volume does not show significant relationship to DR, age, duration of diabetes or the HbA_{1c} levels. Thus the retinal parameters already exhibit the characteristics of a prospective biomarker for diabetic peripheral neuropathy. These findings are novel. As a subsequent step, the visual function needs to be assessed. Thereafter, the retinal thickness and visual functional parameters together require validation as potential biomarkers (Stem et al., 2013) for diabetic peripheral neuropathy.

Validation of the biomarker

The FDA recommends that a potential biomarker needs to be assessed for sensitivity, specificity, reliability and variability of the measurements. Sensitivity and specificity are measures of validity. An example of validity in this context would be if the RTVue OCT measures the correct retinal thickness (validity) and if it is consistent (reliability). A measurement is said to be reliable if it shows minimal variations. Inter- and intra-observer variability indicates the variability in measurements between operators and within individuals,

respectively. Retinal thickness measures have been evaluated for reliability, variability and diagnostic capability as described below. Retinal thickness measures demonstrated high correlation with the histological measurements indicating that the measurements are true retinal tissue thickness (Fukuchi et al., 2001; Knott et al., 2011; Koinzer et al., 2013; Michalewski et al., 2007). The reproducibility of RTVue retinal tissue thickness measures are equivalent to other commercially available instruments (Wolf-Schnurrbusch et al., 2009) and has similar diagnostic capability to other commercial instruments (Leite et al., 2011). Sensitivity indicates the ability of the test to correctly identify the number of people who actually have the disease. Specificity indicates the ability of the test to correctly identify those who don't have the disease. Positive predictive value is the percentage of people with a positive test who actually have the disease. Negative predictive value is the percentage of people with a negative test result who don't have the disease (Parikh et al., 2008). These statistics need to be determined for retinal thickness assessment if such parameters are to serve as valid biomarkers for diabetic peripheral neuropathy.

The current research program has demonstrated the retinal thickness measures are significantly associated with the NDS. As a subsequent step, the relationship with the electrophysiology measures may be assessed; furthermore, the sensitivity, specificity, inter-observer and the intra-observer variability of retinal thickness measurements and the predictive capability assessment in individuals with and without neuropathy and in various stages of neuropathy may be valuable. A receiver operating characteristic curve analysis can be performed to determine the diagnostic capability and validity of retinal tissue thickness measurement and visual function loss.

It is to be noted that there is currently no single, effective, valid means to diagnose diabetic peripheral neuropathy. It is possible that ophthalmic markers may ultimately outperform the current screening methods for DPN and may decrease the need for painful, expensive and invasive procedures such as skin or nerve biopsies (Efron, 2011).

7.4 Future directions of this research

An important follow-on from this study will be a longitudinal study assessing the retinal tissue thickness in relation to the natural history of diabetic neuropathy. This will provide a better understanding of these changes in relation to neural pathology occurring elsewhere in the body in individuals with diabetes. Investigation of other factors such as cardiovascular and nephropathy related factors in relation to retinal tissue thickness may be of interest. The current study may be a foundation to understand the risk of other micro and macrovascular complications of diabetes in relation to retinal tissue thickness. In addition, examination of the relationship between retinal tissue thickness and factors such as smoking and alcohol intake may be useful.

Exploring other cranial nerves in relation to diabetic peripheral neuropathy

Diabetic peripheral neuropathy has been thought as involving the peripheral nerves only, until studies demonstrated that the disease process can extend beyond the peripheral nerves (Eaton et al., 2001). The corneal nerve structure measured as nerve fibre length, count, branching, beading, width, tortuosity, orientation and reflectivity is compromised in relation to diabetic peripheral neuropathy (discussed in Chapter 1). Corneal nerves constitute the ophthalmic division of trigeminal nerve, which is the fifth cranial nerve. This opens up the possibility that diabetic peripheral neuropathy also involves the central nervous system. The RNFL thickness is compromised in relation to diabetic neuropathy (Shahidi et al., 2012) and this finding has been reiterated in the current research program. Consequently, the involvement of the second cranial nerve has been authenticated. It is also likely that the other cranial nerves may be involved in relation to diabetic peripheral neuropathy. The following sections provide an overview of the cranial nerves and methods of clinical assessment.

Testing for the other cranial nerves

Cranial nerve I (Olfactory nerve)

This nerve governs the sense of smell or olfaction. The assessment is performed by asking the patient to identify different aromas. The testing is performed for each nostril separately (Clark et al., 1990).

Cranial nerves II, III, IV and VI

The axons of the retinal nerve fibres represent cranial nerve II. Autonomic neuropathy may manifest as pupillary miosis or sudden extraocular muscle palsy thus indicating the involvement of III (Oculomotor nerve) and possibly IV (Trochlear nerve) and VI (Abducens nerve) cranial nerves (Fite et al., 1990). The extra ocular movements can be tested by the broad H test. Extraocular muscles can be tested for saccades (rapid eye movements) and pursuits (slow following movements) as well as the ability to converge and diverge. The assessment of eye deviations and the ability of the eyes to maintain fixation can be tested with cover tests. Pupillary reflex tests can be performed to evaluate the sympathetic and parasympathetic innervation.

Cranial nerve V (Trigeminal nerve)

The cornea receives its sensory innervation from the ophthalmic division of the trigeminal nerve. The non-ophthalmic component of the trigeminal nerve serves the sensory component of the face as well as motor supply to the muscles of mastication. Tests for sensory innervation include assessment of sensation to light touch and pin prick sensation with cotton wool and pins on both sides of the face along the three divisions of the trigeminal nerve. To examine the muscles of mastication, the patient is asked to clench the jaw and the examiner should feel equal muscle bulk on both sides of the jaw and temple (Walker et al., 1990).

Cranial nerve VII (Facial nerve)

This nerve governs the sensation on the skin of the face, motor control to the muscles in the face and also the anterior 2/3^{rds} of the tongue. Motor control is assessed by simply asking the patient to wrinkle their eyebrows. It should be symmetrical on both sides. The patient is asked to show their teeth and to pop out their cheeks. Facial nerve supplies the orbicularis muscle that helps in closure of the eyelids. The nerve involvement can be tested by Bell's phenomenon; the patient is asked to close his or her eyes tightly while the examiner tries to open the eyes. It is expected that the eye balls should be covered after eye lid closure. Any asymmetry or incomplete closure or lack of resistance to forced opening of the eyelid is abnormal (Walker, 1990). Absence of Bell's phenomenon may lead to exposure of the cornea resulting in exposure keratitis (Brown, 1982).

Cranial nerve VIII (Auditory and Vestibular nerves)

This nerve governs hearing and balance. Weber test involves placing a tuning fork on the forehead. The normal response is to hear the tuning fork in both the ears. Rhine test involves placing a vibrating tuning fork on the mastoid process. The patient is asked to report when they cannot hear anymore. Following this, the other end of the tuning fork is brought to the front of the ear. The tuning fork would still be ringing because the air conduction pathway is more sensitive than the bone conduction pathway. This test can reportedly detect conductive hearing defect (Sanders et al., 2010).

Cranial nerve IX nerve (Glossopharyngeal nerve)

This nerve serves the taste sensation in the posterior 1/3rd of the tongue and also gag reflex. The gag reflex is tested by touching the palate with a tongue blade and observing for ipsilateral tongue contraction (Dodds, 1989).

Cranial nerve X (Vagus nerve)

This nerve serves muscles that aid in swallowing and in parasympathetic responses of the heart. The patient can be given water to drink, as a test for swallowing. Difficulty in swallowing may result in coughing (Dodds, 1989). Parasympathetic heart responses are assessed as beat-beat variations in heart rate with deep breathing, valsalva manoeuvre and postural changes in blood pressure.

Cranial nerve XI (Spinal Accessory nerve)

The nerve supplies the sternomastoid muscle. Testing is performed by asking the patient to turn the head sideways and if they can hold like that against resistance applied with the palm of the examiner. The examiner looks for stiffening of the neck muscle (sternomastoid muscle) on the opposite side (Gillig et al., 2010).

Cranial nerve XII (Hypoglossal nerve)

This nerve aids in motor control of the tongue. The muscle can be tested by asking the person to protrude their tongue and also observing tongue movements to the left and to the right. Evaluation can be as simple as looking inside the mouth of the patient to know if the tongue is steady or fasciculating (Gillig et al., 2010).

Implications for patients with diabetic neuropathy

Individuals with diabetic neuropathy especially with foot complications may have gait problems (Allet et al., 2008; Katoulis et al., 1997) either due to loss of sensation or due to painful symptoms in the feet. This may become significant and more complicated in the presence of reduced visual field sensitivity (Sampson et al., 2012). As a consequence, they are may be more prone to bump into objects especially in dim illumination, making them more susceptible to foot injury or even falls (Gabell et al., 1985; Richardson et al., 1995). Patients can have defective colour vision that interferes with the quality of life, difficulty

in driving and face recognition. Other micro and macrovascular complications and amputation may complicate existing health conditions. For the reason that diabetic peripheral neuropathy is a debilitating disorder for which there is no effective treatment, it is crucial that the disease be screened and identified in its early stages so as to intervene as early as possible, and thereby prevent avoidable complications.

Implications for eye care practitioner

The eye care practitioners must note that the absence of diabetic retinopathy does not rule out any retinal complications. Individuals can have undiagnosed diabetic peripheral neuropathy and its associated neuroretinal complications. Due to the transparent nature of the retinal nerve fibre layer, a compromise to the neural layers may or may not be noticeable during a routine eye examination. Therefore, these individuals need to be referred to their health care provider for appropriate follow-up so as to detect undiagnosed disease or other micro and macrovascular complications of diabetes. This would help in intervening at the early stages so that any avoidable complications can be prevented. Conversely, once these retinal findings have been established as markers, ophthalmic screening may detect a previously undiagnosed neuropathy. The patients can be subsequently referred to a specialist for a comprehensive evaluation.

Implications for endocrinologist

Peripheral neuropathy is a debilitating complication of diabetes for which there is no effective treatment. Therefore, the emphasis here is on prevention or slowing down the progression of neuropathy. Although clinical trials tested aldose reductase inhibitors, antioxidants and nerve growth factors as treatment options for diabetic neuropathy, the results were disappointing (Habib et al., 2010). The only aspect that showed promising outcomes was optimal glycaemic levels. Endocrinologists can play a significant role in working with the patients to achieve optimal glycaemic levels as well as in counselling the patient in recording the everyday levels. In addition, podiatrists can assist by educating

patients about the importance of regular health check-up and proper foot care. Counselling and advice regarding proper fitting shoes and protection of sensitive areas in the feet should be emphasized.

If the various ophthalmic markers of diabetic neuropathy such as the retinal markers investigated in this thesis are eventually validated, then eye care practitioners may become an important part of multidisciplinary teams (Gardner et al., 2011), including diabetic physicians and podiatrists, in identifying and monitoring patients with diabetic peripheral neuropathy. A timely referral and management plan can prevent or delay unnecessary consequences.

Master References

Abbott, N. J., Rönnbäck, L., & Hansson, E. (2006). Astrocyte–endothelial interactions at the blood–brain barrier. *Nature Reviews Neuroscience, 7*(1), 41-53.

Adhi, M., Aziz, S., Muhammad, K., & Adhi, M. I. (2012). Macular thickness by age and gender in healthy eyes using spectral domain optical coherence tomography. *PLoS ONE, 7*(5), e37638.

Adler, A. I., et al. (1997). Risk factors for diabetic peripheral sensory neuropathy: Results of the seattle prospective diabetic foot study. *Diabetes Care, 20*(7), 1162-1167.

Agardh, E. (2002). A case of progression of diabetic retinopathy during pregnancy. *Acta Ophthalmol Scand, 80*(5), 524-530.

Aguilar, E., Friedlander, M., & Gariano, R. F. (2003). Endothelial proliferation in diabetic retinal microaneurysms. *Arch Ophthalmol, 121*(5), 740-741.

Alamouti, B., & Funk, J. (2003). Retinal thickness decreases with age: An oct study. *Br J Ophthalmol, 87*(7), 899-901.

Alkuraya, H., Kangave, D., & Abu El-Asrar, A. M. (2005). The correlation between optical coherence tomographic features and severity of retinopathy, macular thickness and visual acuity in diabetic macular edema. *International ophthalmology, 26*, 93-99.

Allet, L., et al. (2008). Gait characteristics of diabetic patients: A systematic review. *Diabetes Metab Res Rev, 24*(3), 173-191.

American Diabetes Association. (1985). Photocoagulation for diabetic macular edema. Early treatment diabetic retinopathy study report number 1. Early treatment diabetic retinopathy study research group. *Arch Ophthalmol, 103*(12), 1796-1806.

Antonetti, D. A., et al. (2006). Diabetic retinopathy. *Diabetes, 55*(9), 2401-2411.

Antonetti, D. A., Klein, R., & Gardner, T. W. (2012). Diabetic retinopathy. *New Engl J Med, 366*(13), 1227-1239.

Antonetti, D. A., Lieth, E., Barber, A. J., & Gardner, T. W. (1999). Molecular mechanisms of vascular permeability in diabetic retinopathy. *Semin Ophthalmol, 14*(4), 240-248.

Apple, D. J. (1981). Anatomy and histopathology of the macular region. *Int Ophthalmol Clin, 21*(3), 1-9.

Archer, A., Watkins, P., Thomas, P., Sharma, A., & Payan, J. (1983). The natural history of acute painful neuropathy in diabetes mellitus. *Journal of Neurology, Neurosurgery & Psychiatry, 46*(6), 491-499.

Asefzadeh, B., Cavallerano, A. A., & Fisch, B. M. (2007). Racial differences in macular thickness in healthy eyes. *Optom Vis Sci, 84*(10), e941-e945.

Ashton, N. (1949). Vascular changes in diabetes with particular reference to the retinal vessels: Preliminary report. *Br J Ophthalmol, 33*(7), 407-420.

Balasubramanian, M., Bowd, C., Vizzeri, G., Weinreb, R. N., & Zangwill, L. M. (2009). Effect of image quality on tissue thickness measurements obtained with spectral domain-optical coherence tomography. *Optics express, 17*(5), 4019-4036.

Barber, A. J. (2003). A new view of diabetic retinopathy: A neurodegenerative disease of the eye. *Prog Neuro-PsychoPh, 27*(2), 283-290.

Barber, A. J., et al. (1998). Neural apoptosis in the retina during experimental and human diabetes. Early onset and effect of insulin. *Journal of Clinical Investigation, 102*(4), 783-791.

Baseler, H. A., Sutter, E. E., Klein, S. A., & Carney, T. (1994). The topography of visual evoked response properties across the visual field. *Electroencephalography and clinical neurophysiology, 90*(1), 65-81.

Berlien, H.-P. (2003). *Applied laser medicine*: Springer Verlag

Bernard, J., Guyot-Argenton, C., Benmehidi, A., & Marielescure, C. (1995). Hard exudates in diabetic retinopathy. *Bulletin de la Société belge d'ophtalmologie, 256*, 61-64.

Biallosterski, C., et al. (2007). Decreased optical coherence tomography-measured pericentral retinal thickness in patients with diabetes mellitus type 1 with minimal diabetic retinopathy. *Br J Ophthalmol, 91*(9), 1135-1138.

Blankenburg, M., et al. (2012). Childhood diabetic neuropathy: Functional impairment and non-invasive screening assessment. *Diabet Med, 29*(11), 1425-1432.

Blankenship, G. W. (1986). Red krypton and blue-green argon panretinal laser photocoagulation for proliferative diabetic retinopathy: A laboratory and clinical comparison. *T Am Ophthal Soc, 84*, 967-1003.

Bodansky, H., Cudworth, A., Whitelocke, R., & Dobree, J. (1982). Diabetic retinopathy and its relation to type of diabetes: Review of a retinal clinic population. *Br J Ophthalmol, 66*(8), 496-499.

Booya, F., et al. (2005). Potential risk factors for diabetic neuropathy: A case control study. *BMC neurology, 5*(1), 24.

Boulton, A. J. (2012). *Diabetic neuropathy: Is pain god's greatest gift to mankind?* Paper presented at the Seminars in Vascular Surgery

Boulton, A. J. M. (2005). Management of diabetic peripheral neuropathy. *Clin Diabetes, 23*(1), 9-15.

Boulton, A. J. M., & Malik, R. A. (1998). Diabetic neuropathy. *Med Clin North Am, 82*(4), 909-929.

Boulton, A. J. M., Malik, R. A., Arezzo, J. C., & Sosenko, J. M. (2004). Diabetic somatic neuropathies. *Diabetes Care, 27*(6), 1458-1486.

Boulton, A. J. M., & Vileikyte, L. (2009). Diagnosis and staging. *Managing Neuropathic Pain in the Diabetic Patient*, 21-33.

Bressler, N. M., et al. (2008). Retinal thickness on stratus optical coherence tomography in people with diabetes and minimal or no diabetic retinopathy. *Am J Ophthalmol, 145*(5), 894-901.

Bringmann, A., Grosche, A., Pannicke, T., & Reichenbach, A. (2013). Gaba and glutamate uptake and metabolism in retinal glial (müller) cells. *Frontiers in Endocrinology, 4*.

Bronson-Castain, K. W., et al. (2009). Adolescents with type 2 diabetes: Early indications of focal retinal neuropathy, retinal thinning and venular dilation. *Retina (Philadelphia, Pa.), 29*(5), 618.

Brown, J. S. (1982). Bell's palsy: A 5 year review of 174 consecutive cases: An attempted double blind study. *Laryngoscope, 92*(12), 1369-1373.

Brown, M. J., & Asbury, A. K. (2004). Diabetic neuropathy. *Annals of neurology, 15*(1), 2-12.

Browning, D. J., Fraser, C. M., & Clark, S. (2008). The relationship of macular thickness to clinically graded diabetic retinopathy severity in eyes without clinically detected diabetic macular edema. *Ophthalmology, 115*(3), 533-539. e532.

Budenz, D. L., et al. (2007). Determinants of normal retinal nerve fiber layer thickness measured by stratus oct. *Ophthalmology, 114*(6), 1046-1052.

Bursell, S.-E., et al. (1996). Retinal blood flow changes in patients with insulin-dependent diabetes mellitus and no diabetic retinopathy. *Invest Ophthalmol Vis Sci, 37*(5), 886-897.

Cameron, N. E., Eaton, S. E. M., Cotter, M. A., & Tesfaye, S. (2001). Vascular factors and metabolic interactions in the pathogenesis of diabetic neuropathy. *Diabetologia, 44*(11), 1973-1988.

Cankaya, A. B., Ozdamar, Y., Ozalp, S., & Ozkan, S. S. (2011). Impact of panretinal photocoagulation on optic nerve head parameters. *Ophthalmologica, 225*(4), 193-199.

Chan, A., Duker, J. S., Ko, T. H., Fujimoto, J. G., & Schuman, J. S. (2006). Normal macular thickness measurements in healthy eyes using stratus optical coherence tomography. *Arch Ophthalmol, 124*(2), 193-198.

Chen, Y., et al. (2001). Astrocytes protect neurons from nitric oxide toxicity by a glutathione-dependent mechanism. *Journal of neurochemistry, 77*(6), 1601-1610.

Chihara, E., & Zhang, S. (1998). Analysis of diabetic optic neuropathy with a topographic laser scanning system. *Nippon Ganka Gakkai Zasshi, 102*(7), 431-435.

Cho, H. Y., Lee, D. H., Chung, S. E., & Kang, S. W. (2010). Diabetic retinopathy and peripapillary retinal thickness. *Korean J Ophthalmol, 24*(1), 16-22.

Clark, V. L., & Kruse, J. A. (1990). Clinical methods: The history, physical, and laboratory examinations. *JAMA, 264*(21), 2808-2809.

Cole, B. L. (2004). The handicap of abnormal colour vision. *Clin Exp Optom, 87*(4-5), 258-275.

Coupland, S. G. (1987). A comparison of oscillatory potential and pattern electroretinogram measures in diabetic retinopathy. *Doc Ophthalmol, 66*(3), 207-218.

Craitoiu, S., Mocanu, C., Olaru, C., & Rodica, M. (2005). [retinal vascular lesions in diabetic retinopathy]. *Oftalmologia (Bucharest, Romania : 1990), 49*(1), 82-87.

Curcio, C. A., & Allen, K. A. (1990). Topography of ganglion cells in human retina. *Journal of Comparative Neurology, 300*(1), 5-25.

Dandona, L., Dandona, R., Naduvilath, T. J., McCarty, C. A., & Rao, G. N. (1999). Population based assessment of diabetic retinopathy in an urban population in southern india. *Br J Ophthalmol, 83*(8), 937-940.

Daube., J. R., & Rubin., D. I. (2009). *Clinical neurophysiology*: Oxford University Press, USA

David, E. J., & Ohio James, M. F. (2005). Evaluation and prevention of diabetic neuropathy. *Am Fam Physician, 71*(11), 2123-2128.

Davis, M. D. (1992). Diabetic retinopathy: A clinical overview. *Diabetes Care, 15*(12), 1844-1874.

Davis, M. D., et al. (1998). Risk factors for high-risk proliferative diabetic retinopathy and severe visual loss: Early treatment diabetic retinopathy study report #18. *Invest Ophthalmol Vis Sci, 39*(2), 233-252.

Davis, M. D., Norton, E. W. D., & Myers, F. (1969). *The airlie classification of diabetic retinopathy*

de Boer, M. R., et al. (2005). Better lighting to reduce falls and fracture? *Journal of Bone and Mineral Research, 20*(11), 2063-2063.

de Oliveira, F. (1966). Pericytes in diabetic retinopathy. *Br J Ophthalmol, 50*(3), 134-143.

Demb, J. B., Boynton, G. M., & Heeger, D. J. (1997). Brain activity in visual cortex predicts individual differences in reading performance. *Proceedings of the National Academy of Sciences, 94*(24), 13363-13366.

Derrington, A., & Lennie, P. (1984). Spatial and temporal contrast sensitivities of neurones in lateral geniculate nucleus of macaque. *J Physiol, 357*(1), 219-240.

Dodds, W. J. (1989). The physiology of swallowing. *Dysphagia, 3*(4), 171-178.

Dros, J., Wewerinke, A., Bindels, P. J., & van Weert, H. C. (2009). Accuracy of monofilament testing to diagnose peripheral neuropathy: A systematic review. *The Annals of Family Medicine, 7*(6), 555-558.

Eaton, S. E. M., et al. (2001). Spinal-cord involvement in diabetic peripheral neuropathy. *The Lancet, 358*(9275), 35-36.

Edwards, K., et al. (2012). Wide-field assessment of the human corneal subbasal nerve plexus in diabetic neuropathy using a novel mapping technique. *Cornea, 31*(9), 1078-1082.

Edwards, K., et al. (2012). Utility of corneal confocal microscopy for assessing mild diabetic neuropathy: Baseline findings of the landmark study. *Clin Exp Optom, 95*(3), 348-354.

Efron, N. (2011). The glenn a. Fry award lecture 2010: Ophthalmic markers of diabetic neuropathy. *Optom. Vis. Sci., 88*(6), 661-683.

Efron, N., et al. (2010). Repeatability of measuring corneal subbasal nerve fiber length in individuals with type 2 diabetes. *Eye & contact lens, 36*(5), 245-248.

Ehrlich, R., et al. (2010). Diabetic macular oedema: Physical, physiological and molecular factors contribute to this pathological process. *Acta Ophthalmol, 88*(3), 279-291.

Eriksson, U., & Alm, A. (2009). Macular thickness decreases with age in normal eyes: A study on the macular thickness map protocol in the stratus oct. *Br J Ophthalmol, 93*(11), 1448-1452.

Ewing, D., & Clarke, B. (1982). Diagnosis and management of diabetic autonomic neuropathy. *Brit Med J, 285*(6346), 916-918.

Falsini, B., et al. (1989). Steady-state pattern electroretinogram in insulin-dependent diabetics with no or minimal retinopathy. *Doc Ophthalmol, 73*(2), 193-200.

Fantes, F. E., & Anderson, D. R. (1989). Clinical histologic correlation of human peripapillary anatomy. *Ophthalmology, 96*(1), 20-25.

Farnsworth, D., & Color, M. (1957). *The farnsworth-munsell 100-hue test: For the examination of color discrimination*: Munsell Color Company

Feit-Leichman, R. A., et al. (2005). Vascular damage in a mouse model of diabetic retinopathy: Relation to neuronal and glial changes. *Invest Ophthalmol Vis Sci, 46*(11), 4281-4287.

Ferris, F. L., Chew, E. Y., & Hoogwerf, B. J. (1996). Serum lipids and diabetic retinopathy. *Diabetes Care, 19*(11), 1291-1293.

Figueira, J., et al. (2009). Prospective randomised controlled trial comparing sub-threshold micropulse diode laser photocoagulation and conventional green laser for clinically significant diabetic macular oedema. *Br J Ophthalmol, 93*(10), 1341-1344.

Fite, J. D., & Walker, H. K. (1990). Cranial nerves iii, iv, and vi: The oculomotor, trochlear, and abducens nerves. *Clinical methods: the history, physical, and laboratory examinations*, 308.

Fletcher, E. L., Phipps, J. A., & Wilkinson-Berka, J. L. (2005). Dysfunction of retinal neurons and glia during diabetes. *Clin Exp Optom, 88*(3), 132-145.

Forrest, K. Y.-Z., Maser, R. E., Pambianco, G., Becker, D. J., & Orchard, T. J. (1997). Hypertension as a risk factor for diabetic neuropathy: A prospective study. *Diabetes, 46*(4), 665-670.

Fortune, B., Demirel, S., Zhang, X., Hood, D. C., & Johnson, C. A. (2006). Repeatability of normal multifocal vep: Implications for detecting progression. *J Glaucoma, 15*(2), 131-141.

Fortune, B., Schneck, M. E., & Adams, A. J. (1999). Multifocal electroretinogram delays reveal local retinal dysfunction in early diabetic retinopathy. *Invest Ophthalmol Vis Sci, 40*(11), 2638-2651.

Fox, C. S. (2010). Cardiovascular disease risk factors, type 2 diabetes mellitus, and the framingham heart study. *Trends in cardiovascular medicine, 20*(3), 90-95.

Framme, C., Schuele, G., Roider, J., Birngruber, R., & Brinkmann, R. (2004). Influence of pulse duration and pulse number in selective rpe laser treatment. *Lasers in surgery and medicine, 34*(3), 206-215.

Fraser-Bell, S., et al. (2005). Age, gender, ethnicity and refractive error related differences in normal macular thickness and volume as measured by stratus oct. *Invest. Ophthalmol. Vis. Sci., 46*(5), E-Abstract 1542. Retrieved from http://abstracts.iovs.org/cgi/content/abstract/1546/1545/1542.

Freeman, E. E., Muñoz, B., Rubin, G., & West, S. K. (2007). Visual field loss increases the risk of falls in older adults: The salisbury eye evaluation. *Invest Ophthalmol Vis Sci, 48*(10), 4445-4450.

Fukuchi, T., Takahashi, K., & Shou, K. (2001). Optical coherence tomography (oct) findings in normal retina and laser-induced choroidal neovascularization in rats. *Graefes Arch Clin Exp Ophthalmol, 239*(1), 41-46.

Gabell, A., Simons, M. A., & Nayak, U. S. L. (1985). Falls in the healthy elderly: Predisposing causes. *Ergonomics, 28*(7), 965-975.

Garas, A., Papp, A., & Holló, G. (2012). Influence of age-related macular degeneration on macular thickness measurement made with fourier-domain optical coherence tomography. *J Glaucoma, 22*(3), 195-200.

Gardner, T. W., Abcouwer, S. F., Barber, A. J., & Jackson, G. R. (2011). An integrated approach to diabetic retinopathy research. *Arch Ophthalmol, 129*(2), 230-235.

Gardner, T. W., Antonetti, D. A., Barber, A. J., LaNoue, K. F., & Levison, S. W. (2002). Diabetic retinopathy: More than meets the eye. *Surv Ophthalmol, 47, Supplement 2*(0), S253-S262.

Gardner, T. W., et al. (1997). Astrocytes increase barrier properties and zo-1 expression in retinal vascular endothelial cells. *Invest Ophthalmol Vis Sci, 38*(11), 2423-2427.

Gariano, R. F., Sage, E. H., Kaplan, H. J., & Hendrickson, A. E. (1996). Development of astrocytes and their relation to blood vessels in fetal monkey retina. *Invest Ophthalmol Vis Sci, 37*(12), 2367-2375.

Gaucher, D., et al. (2007). Microglial changes occur without neural cell death in diabetic retinopathy. *Vision Res, 47*(5), 612-623.

Georgakopoulos, C. D., et al. (2011). Decreased contrast sensitivity in children and adolescents with type 1 diabetes mellitus. *J Pediat Ophth Strab, 48*(3), 92-97.

Ghirlanda, G., et al. (1991). Detection of inner retina dysfunction by steady-state focal electroretinogram pattern and flicker in early iddm. *Diabetes, 40*(9), 1122-1127.

Gillig, P. M., & Sanders, R. D. (2010). Cranial nerves ix, x, xi, and xii. *Psychiatry (Edgmont), 7*(5), 37.

Goebel, W., & Kretzchmar-Gross, T. (2002). Retinal thickness in diabetic retinopathy: A study using optical coherence tomography (oct). *Retina, 22*(6), 759-767.

Goldstein, B. J., & Mueller-Wieland, D. (2013). *Type 2 diabetes: Principles and practice, second edition*: Taylor & Francis

Green, F., et al. (1985). Colour vision of diabetics. *Br J Ophthalmol, 69*(7), 533-536.

Grunwald, J., Brucker, A., Petrig, B., & Riva, C. (1989). Retinal blood flow regulation and the clinical response to panretinal photocoagulation in proliferative diabetic retinopathy. *Ophthalmology, 96*(10), 1518-1522.

Grunwald, J., Riva, C. E., Brucker, A. J., Sinclair, S., & Petrig, B. (1986). Effect of panretinal photocoagulation on retinal blood flow in proliferative diabetic retinopathy. *Ophthalmology, 93*(5), 590.

Habib, A. A., & Brannagan III, T. H. (2010). Therapeutic strategies for diabetic neuropathy. *Current neurology and neuroscience reports, 10*(2), 92-100.

Hardy, K., Lipton, J., Scase, M., Foster, D., & Scarpello, J. (1992). Detection of colour vision abnormalities in uncomplicated type 1 diabetic patients with angiographically normal retinas. *Br J Ophthalmol, 76*(8), 461-464.

Harris Nwanyanwu, K., et al. (2013). Predicting development of proliferative diabetic retinopathy. *Diabetes Care, 36*(6), 1562-1568.

Hartridge, H. (1922). Visual acuity and the resolving power of the eye. *J Physiol, 57*(1-2), 52-67.

Harwerth, R. S., Carter-Dawson, L., Shen, F., Smith, E. L., & Crawford, M. L. J. (1999). Ganglion cell losses underlying visual field defects from experimental glaucoma. *Invest Ophthalmol Vis Sci, 40*(10), 2242-2250.

Harwerth, R. S., et al. (2004). Neural losses correlated with visual losses in clinical perimetry. *Invest Ophthalmol Vis Sci, 45*(9), 3152-3160.

Henricsson, M., & Heijl, A. (1994). Visual fields at different stages of diabetic retinopathy. *Acta Ophthalmol, 72*(5), 560-569.

Heravian, J., et al. (2010). Evaluation of contrast sensitivity, color vision and visual acuity in patients with and without diabetes. *Iran. J. Ophthalmol., 22*(3), 33-40.

Hildebrand, G. D., & Fielder, A. R. (2010). Anatomy and physiology of the retina. *Pediatric Retina*, 39-65.

Hoffmann, M. B. (2008). Investigating visual function with multifocal visual evoked potentials *Pediatric ophthalmology, neuro-ophthalmology, genetics* (pp. 139-159): Springer.

Hogan, M. J., & Feeney, L. (1963). The ultrastructure of the retinal vessels: Iii. Vascular-glial relationships. *Journal of ultrastructure research, 9*(1), 47-64.

Holder, G. E., Brigell, M. G., Hawlina, M., Meigen, T., & Bach, M. (2007). Iscev standard for clinical pattern electroretinography—2007 update. *Documenta ophthalmologica, 114*(3), 111-116.

Hood, D. C. (2000). Assessing retinal function with the multifocal technique. *Prog Retin Eye Res, 19*(5), 607-646.

Hood, D. C., et al. (2012). Iscev standard for clinical multifocal electroretinography (mferg)(2011 edition). *Documenta Ophthalmologica, 124*(1), 1-13.

Hood, D. C., et al. (2008). Iscev guidelines for clinical multifocal electroretinography (2007 edition). *Documenta Ophthalmologica, 116*(1), 1-11.

Horowitz, S. H. (2002). Criteria for the diagnosis of peripheral neuropathies. *Occupational and Environmental Medicine, 59*(7), 425-426.

Hsieh, S.-T. (2010). Pathology and functional diagnosis of small-fiber painful neuropathy. *Acta Neurol Taiwan, 19*, 82-89.

Hsu, S. Y., & Chung, C. P. (2002). Evaluation of retinal nerve fiber layer thickness in diabetic retinopathy after panretinal photocoagulation. *The Kaohsiung journal of medical sciences, 18*(8), 397-400.

Huang, J., et al. (2009). Macular thickness measurements in normal eyes with time domain and fourier domain optical coherence tomography. *Retina (Philadelphia, Pa.), 29*(7), 980-987.

Hulka, B. S. (1991). Epidemiological studies using biological markers: Issues for epidemiologists. *Cancer Epidemiol Biomarkers Prev, 1*(1), 13-19.

HyvÄRinen, L. E. A., Laurinen, P., & Rovamo, J. (1983). Contrast sensitivity in evaluation of visual impairment due to diabetes. *Acta Ophthalmol, 61*(1), 94-101.

Imai, H., Singh, R. S., Fort, P. E., & Gardner, T. W. (2009). Neuroprotection for diabetic retinopathy. *Developments in ophthalmology, 44*, 56-68.

Ismail, G. M., & Whitaker, D. (1998). Early detection of changes in visual function in diabetes mellitus. *Ophthal Physl Opt, 18*(1), 3-12.

Jackson, G. R., Scott, I. U., Quillen, D. A., Walter, L. E., & Gardner, T. W. (2012). Inner retinal visual dysfunction is a sensitive marker of non-proliferative diabetic retinopathy. *Br J Ophthalmol, 96*(5), 699-703.

Jain, K. K. (2010). *The handbook of biomarkers*. New York: Springer

Jamal, G. A., Hansen, S., Weir, A. I., & Ballantyne, J. P. (1987). The neurophysiologic investigation of small fiber neuropathies. *Muscle & nerve, 10*(6), 537-545.

Jiang, L., et al. (2011). Application of multifocal visual evoked potentials in the assessment of visual dysfunction in macular diseases. *Eye (Lond), 25*(10), 1302-1309.

Johns, K., Leonard-Martin, T., & Feman, S. (1989). The effect of panretinal photocoagulation on optic nerve cupping. *Ophthalmology, 96*(2), 211-216.

Johnson, C. A., & Keltner, J. L. (1983). Incidence of visual field loss in 20,000 eyes and its relationship to driving performance. *Arch Ophthalmol, 101*(3), 371-375.

Joslin, E. P., Hoot, H. F., White, P., & Marble, A. (1936). The treatment of diabetes mellitus. *Am J Med Sci, 191*(1), 127-130.

Kallinikos, P., et al. (2004). Corneal nerve tortuosity in diabetic patients with neuropathy. *Invest Ophthalmol Vis Sci, 45*(2), 418-422.

Kamiya, H., Murakawa, Y., Zhang, W., & Sima, A. A. F. (2005). Unmyelinated fiber sensory neuropathy differs in type 1 and type 2 diabetes. *Diabetes Metab Res Rev, 21*(5), 448-458.

Kashani, A. H., et al. (2010). Retinal thickness analysis by race, gender, and age using stratus oct. *Am J Ophthalmol, 149*(3), 496-502.

Katoulis, E. C., et al. (1997). Gait abnormalities in diabetic neuropathy. *Diabetes Care, 20*(12), 1904-1907.

Kelly, D. (1966). Frequency doubling in visual responses. *JOSA, 56*(11), 1628-1632.

Kelty, P. J., et al. (2008). Macular thickness assessment in healthy eyes based on ethnicity using stratus oct optical coherence tomography. *Invest Ophthalmol Vis Sci, 49*(6), 2668-2672.

Kempen, J. H., et al. (2004). The prevalence of diabetic retinopathy among adults in the united states. *Arch Ophthalmol, 122*(4), 552-563.

Kern, T. S., & Barber, A. J. (2008). Retinal ganglion cells in diabetes. *J Physiol, 586*(18), 4401-4408.

Kim, H. Y., & Cho, H. K. (2009). Peripapillary retinal nerve fiber layer thickness change after panretinal photocoagulation in patients with diabetic retinopathy. *Korean J Ophthalmol, 23*(1), 23-26.

Kim, N. R., et al. (2011). Determinants of perimacular inner retinal layer thickness in normal eyes measured by fourier-domain optical coherence tomography. *Invest Ophthalmol Vis Sci, 52*(6), 3413-3418.

Kim, T.-W., Park, U.-C., Park, K. H., & Kim, D. M. (2007). Ability of sratus oct to identify localized retinal nerve fiber layer defects in patients with normal standard automated perimetry results. *Invest Ophthalmol Vis Sci, 48*(4), 1635-1641.

Klein, R., Klein, B. K., Moss, S. E., Davis, M. D., & DeMets, D. L. (1988). Glycosylated hemoglobin predicts the incidence and progression of diabetic retinopathy. *JAMA, 260*(19), 2864-2871.

Klistorner, A., & Graham, S. (2005). Intertest variability of mfvep amplitude: Reducing its effect on the interpretation of sequential tests. *Documenta ophthalmologica, 111*(3), 159-167.

Klistorner, A. I., Graham, S. L., Grigg, J. R., & Billson, F. A. (1998). Multifocal topographic visual evoked potential: Improving objective detection of local visual field defects. *Invest Ophthalmol Vis Sci, 39*(6), 937-950.

Knott, E. J., Sheets, K. G., Zhou, Y., Gordon, W. C., & Bazan, N. G. (2011). Spatial correlation of mouse photoreceptor-rpe thickness between sd-oct and histology. *Exp Eye Res, 92*(2), 155-160.

Koh, V. T., et al. (2012). Determinants of ganglion cell–inner plexiform layer thickness measured by high-definition optical coherence tomography. *Invest Ophthalmol Vis Sci, 53*(9), 5853-5859.

Kohner, E. M. (1978). The evolution and natural history of diabetic retinopathy. *Int Ophthalmol Clin, 18*(4), 1-16.

Koinzer, S., et al. (2013). Correlation with oct and histology of photocoagulation lesions in patients and rabbits. *Acta Ophthalmol, 91*(8), e603-e611.

Königsreuther, K. A., & Jonas, J. B. (1995). Optic disc morphology in diabetes mellitus. *Graefes Arch Clin Exp Ophthalmol, 233*(4), 200-204.

Krebs, W., & Krebs, I. (1991). Microscopic anatomy *Primate retina and choroid* (pp. 4-9): Springer New York.

Lacomis, D. (2002). Small-fiber neuropathy. *Muscle & nerve, 26*(2), 173-188.

Lam, D. S. C., et al. (2007). Regional variations in the relationship between macular thickness measurements and myopia. *Invest Ophthalmol Vis Sci, 48*(1), 376-382.

Lanting, P., et al. (1990). Assessment of pupillary light reflex latency and darkness adapted pupil size in control subjects and in diabetic patients with and without cardiovascular autonomic neuropathy. *Journal of Neurology, Neurosurgery & Psychiatry, 53*(10), 912-914.

Lasansky, A. (1965). Functional implications of structural findings in retinal glial cells. *Progr. Brain Res, 15*, 48-72.

Lattanzio, R., et al. (2002). Macular thickness measured by optical coherence tomography (oct) in diabetic patients. *Eur J Ophthalmol, 12*(6), 482-487.

Lee, J. E., et al. (2002). Effect of variable tinted spectacle lenses on visual performance in control subjects. *The CLAO journal : official publication of the Contact Lens Association of Ophthalmologists, Inc, 28*(2), 80-82.

Lee, S. B., Kwag, J. Y., Lee, H. J., Jo, Y. J., & Kim, J. Y. (2012). The longitudinal changes of retinal nerve fiber layer thickness after panretinal photocoagulation in diabetic retinopathy patients. *Retina, 33*(1), 188-193.

Leite, M. T., Rao, H. L., Zangwill, L. M., Weinreb, R. N., & Medeiros, F. A. (2011). Comparison of the diagnostic accuracies of the spectralis, cirrus, and rtvue optical coherence tomography devices in glaucoma. *Ophthalmology*.

Lens, A., Nemeth, S. C., & Ledford, J. K. (2008). *Ocular anatomy and physiology*: SLACK

Lieth, E., Gardner, T. W., Barber, A. J., & Antonetti, D. A. (2000). Retinal neurodegeneration: Early pathology in diabetes. *Clin Experiment Ophthalmol, 28*(1), 3-8.

Lim, M. C., et al. (2009). Effect of diabetic retinopathy and panretinal photocoagulation on retinal nerve fiber layer and optic nerve appearance. *Arch Ophthalmol, 127*(7), 857-862.

Lima, V. C., et al. (2010). A comparison between microperimetry and standard achromatic perimetry of the central visual field in eyes with glaucomatous paracentral visual-field defects. *Br J Ophthalmol, 94*(1), 64-67.

Lott, M. E., et al. (2013). Impaired retinal vasodilator responses in prediabetes and type 2 diabetes. *Acta Ophthalmol, 91*(6), e462-469.

Lövestam-Adrian, M., Gränse, L., Andersson, G., & Andreasson, S. (2012). Multifocal visual evoked potentials (mfvep) in diabetic patients with and without polyneuropathy. *The Open Ophthalmology Journal, 6*, 98-103.

Mahon, W., Mitchell, M., Steinke, J., & Raben, M. (1971). Microaneurysms in diabetic retinopathy. *Brit Med J*, 548-549.

Malik, A., Singh, M., Arya, S., Sood, S., & Ichhpujani, P. (2012). Retinal nerve fiber layer thickness in indian eyes with optical coherence tomography. *Nepalese Journal of Ophthalmology, 4*(1), 59-63.

Malik, R. A., et al. (2003). Corneal confocal microscopy: A non-invasive surrogate of nerve fibre damage and repair in diabetic patients. *Diabetologia, 46*(5), 683-688.

Malik, R. A., Kumar, S., & Boulton, A. (1995). Mendenhall's syndrome: Clues to the aetiology of human diabetic neuropathy. *Journal of Neurology, Neurosurgery & Psychiatry, 58*(4), 493-495.

Mäntyjärvi, M., & Laitinen, T. (2001). Normal values for the pelli-robson contrast sensitivity test. *Journal of Cataract & Refractive Surgery, 27*(2), 261-266.

Margrain, T. H., & Thomson, D. (2002). Sources of variability in the clinical photostress test. *Ophthal Physl Opt, 22*(1), 61-67.

Marshall, J. (1970). Thermal and mechanical mechanisms in laser damage to the retina. *Invest Ophthalmol Vis Sci, 9*(2), 97-115.

Marshall, J. (1973). Laser damage to ocular tissues. *Proceedings of the Royal Society of Medicine, 66*(9), 842-844.

Martin, P. M., Roon, P., Van Ells, T. K., Ganapathy, V., & Smith, S. B. (2004). Death of retinal neurons in streptozotocin-induced diabetic mice. *Invest Ophthalmol Vis Sci, 45*(9), 3330-3336.

Massin, P., et al. (2002). Retinal thickness in healthy and diabetic subjects measured using optical coherence tomography mapping software. *Eur J Ophthalmol, 12*(2), 102-108.

Matsuura, H., Setogawa, T., & Tamai, A. (1976). Electron microscopic studies on retinal capillaries in human diabetic retinopathy. *Yonago acta medica, 20*(1), 7-10.

Mayeux, R. (2004). Biomarkers: Potential uses and limitations. *NeuroRx, 1*(2), 182-188.

Mehra, S., et al. (2007). Corneal confocal microscopy detects early nerve regeneration after pancreas transplantation in patients with type 1 diabetes. *Diabetes Care, 30*(10), 2608-2612.

Meijer, J., et al. (2002). Symptom scoring systems to diagnose distal polyneuropathy in diabetes: The diabetic neuropathy symptom score. *Diabet Med, 19*(11), 962-965.

Michalewski, J., Michalewska, Z., Cisiecki, S., awomir, & Nawrocki, J. (2007). Morphologically functional correlations of macular pathology connected with epiretinal membrane formation in spectral optical coherence tomography (soct). *Graefes Arch Clin Exp Ophthalmol, 245*(11), 1623-1631.

Milam, A. H., Li, Z.-Y., & Fariss, R. N. (1998). Histopathology of the human retina in retinitis pigmentosa. *Prog Retin Eye Res, 17*(2), 175-206.

Milder, D. G. (2006). Progression of disease and a remedy: Causative role of macrophages and microglia: Remedial effect of immunomodulatory and immunosuppressive therapies “in combination”. *Medical hypotheses, 67*(4), 736-739.

Mitchell, P., et al. (2008). *Guidelines for the management of diabetic retinopathy*. Retrieved from http://www.nhmrc.gov.au/_files_nhmrc/_gsdata_/_saved_/file/publications/synopses/di15.pdf.

Mizutani, M., Gerhardinger, C., & Lorenzi, M. (1998). Müller cell changes in human diabetic retinopathy. *Diabetes, 47*(3), 445-449.

Mohamed, Q., Gillies, M. C., & Wong, T. Y. (2007). Management of diabetic retinopathy. *Am J Med Sci, 298*(8), 902-916.

Moloney, J., & Drury, M. (1982). The effect of pregnancy on the natural course of diabetic retinopathy. *Am J Ophthalmol, 93*(6), 745-756.

Moon, S. W., et al. (2011). The change of macular thickness measured by optical coherence tomography in relation to glycemic control in diabetic patients. *Graefes Arch Clin Exp Ophthalmol, 249*(6), 839-848.

Muqit, M., Wakely, L., Stanga, P., Henson, D., & Ghanchi, F. (2009). Effects of conventional argon panretinal laser photocoagulation on retinal nerve fibre layer and driving visual fields in diabetic retinopathy. *Eye (Lond), 24*(7), 1136-1142.

Narendran, V., et al. (2002). Diabetic retinopathy among self reported diabetics in southern india: A population based assessment. *Br J Ophthalmol, 86*(9), 1014-1018.

Nasralah, Z., Robinson, W., Jackson, G., & Barber, A. (2013). Measuring visual function in diabetic retinopathy: Progress in basic and clinical research. *J Clin Exp Ophthalmol, 4*(306), 2.

Neely, K. A., Quillen, D. A., Schachat, A. P., Gardner, T. W., & Blankenship, G. W. (1998). Diabetic retinopathy. *Med Clin North Am, 82*(4), 847-876.

Neitz, M., & Neitz, J. (2000). Molecular genetics of color vision and color vision defects. *Arch Ophthalmol, 118*(5), 691-700.

Nema, H. V., Nema, N., Goes, F., & Gopal, L. (2010). *Recent advances in ophthalmology*: Jaypee Brothers, Medical Publishers

Neubauer, A. S., & Ulbig, M. W. (2007). Laser treatment in diabetic retinopathy. *Ophthalmologica, 221*(2), 95-102.

Neveu, M. M., Tufail, A., Dowler, J. G., & Holder, G. E. (2006). A comparison of pattern and multifocal electroretinography in the evaluation of age-related macular degeneration and its treatment with photodynamic therapy. *Documenta ophthalmologica, 113*(2), 71-81.

Newman, E. A. (1987). The muller cell. *Astrocytes, 1*, 149-171.

Nielsen, N. V. (1978). Corneal sensitivity and vibratory perception in diabetes mellitus. *Acta Ophthalmol, 56*(3), 406-411.

Nilsson, M., Von Wendt, G., Wanger, P., & Martin, L. (2007). Early detection of macular changes in patients with diabetes using rarebit fovea test and optical coherence tomography. *Br J Ophthalmol, 91*(12), 1596-1598.

Nitta, K., Saito, Y., Kobayashi, A., & Sugiyama, K. (2006). Influence of clinical factors on blue-on-yellow perimetry for diabetic patients without retinopathy: Comparison with white-on-white perimetry. *Retina, 26*(7), 797-802.

Ong, G., Ripley, L., Newsom, R., & Casswell, A. (2003). Assessment of colour vision as a screening test for sight threatening diabetic retinopathy before loss of vision. *Br J Ophthalmol, 87*(6), 747-752.

Ooto, S., et al. (2011). Effects of age, sex, and axial length on the three-dimensional profile of normal macular layer structures. *Invest Ophthalmol Vis Sci, 52*(12), 8769-8779.

Oshima, Y., Emi, K., Yamanishi, S., & Motokura, M. (1999). Quantitative assessment of macular thickness in normal subjects and patients with diabetic retinopathy by scanning retinal thickness analyser. *Br J Ophthalmol, 83*(1), 54-61.

Oshitari, T., Hanawa, K., & Adachi-Usami, E. (2009). Changes of macular and rnfl thicknesses measured by stratus oct in patients with early stage diabetes. *Eye (Lond), 23*(4), 884-889.

Osterhues, H., Grossmann, G., Kochs, M., & Hombach, V. (1998). Heart-rate variability for discrimination of different types of neuropathy in patients with insulin-dependent diabetes mellitus. *J Endocrinol Invest, 21*(1), 24-30.

Owsley, C., Ball, K., & Keeton, D. M. (1995). Relationship between visual sensitivity and target localization in older adults. *Vision Res, 35*(4), 579-587.

Owsley, C., & McGwin, G. (1999). Vision impairment and driving. *Surv Ophthalmol, 43*(6), 535-550.

Ozdek, S., Lonneville, Y. H., Onol, M., Yetkin, I., & Hasanreisolu, B. B. (2002). Assessment of nerve fiber layer in diabetic patients with scanning laser polarimetry. *Eye (Lond), 16*(6), 761-765.

Parikh, R., Mathai, A., Parikh, S., Sekhar, G. C., & Thomas, R. (2008). Understanding and using sensitivity, specificity and predictive values. *Indian J Ophthalmol, 56*(1), 45-50.

Park, H. Y. L., Kim, I. T., & Park, C. K. (2011). Early diabetic changes in the nerve fibre layer at the macula detected by spectral domain optical coherence tomography. *Br J Ophthalmol, 95*, 1223-1228.

Partanen, J., et al. (1995). Natural history of peripheral neuropathy in patients with non-insulin-dependent diabetes mellitus. *New Engl J Med, 333*(2), 89-94.

Pearson, A. R., Tanner, V., Keightley, S. J., & Casswell, A. G. (1998). What effect does laser photocoagulation have on driving visual fields in diabetics? *Eye (Lond), 12*(1), 64-68.

Pelli, D., & Robson, J. (1988). *The design of a new letter chart for measuring contrast sensitivity.* Paper presented at the Clinical Vision Sciences

Peng, P. H., Lin, H. S., & Lin, S. (2009). Nerve fibre layer thinning in patients with preclinical retinopathy. *Canadian Journal of Ophthalmology/Journal Canadien d'Ophtalmologie, 44*(4), 417-422.

Perkins, B. A., et al. (2010). Prediction of incident diabetic neuropathy using the monofilament examination a 4-year prospective study. *Diabetes Care, 33*(7), 1549-1554.

Pirie, A., & Van Heyningen, R. (1956). *Biochemistry of the eye*: Thomas

Polyak, S. L. (1941). *The retina*: Chic. U.P.

Polydefkis, M., Hauer, P., Griffin, J., & McArthur, J. (2001). Skin biopsy as a tool to assess distal small fiber innervation in diabetic neuropathy. *Diabetes technology & therapeutics, 3*(1), 23-28.

Porta, M., & Bandello, F. (2002). Diabetic retinopathy. *Diabetologia, 45*(12), 1617-1634.

Prager, T. C., Garcia, C. A., Mincher, C. A., Mishra, J., & Chu, H. H. (1990). The pattern electroretinogram in diabetes. *Am J Ophthalmol, 109*(3), 279-284.

Pritchard, N., et al. (in press). Longitudinal assessment of neuropathy in type 1 diabetes using novel ophthalmic markers (landmark): Study design and baseline characteristics. *Diabetes Res Clin Pract*.

Pritchard, N., et al. (2012). Corneal sensitivity is related to established measures of diabetic peripheral neuropathy. *Clin Exp Optom, 95*(3), 355-361.

Pritchard, N., et al. (2010). Corneal sensitivity as an ophthalmic marker of diabetic neuropathy. *Optom. Vis. Sci., 87*(12), 1003-1008.

Prskavec, F. H., Fulmek, R., Klemen, C., & Stelzer, N. (1986). Changes in the visual field and dark adaptation following panretinal photocoagulation in diabetic retinopathy. *Klin Monbl Augenheilkd, 189*(5), 385-387.

Puell, M. C., Palomo, C., Sánchez-Ramos, C., & Villena, C. (2004). Mesopic contrast sensitivity in the presence or absence of glare in a large driver population. *Graefes Arch Clin Exp Ophthalmol, 242*(9), 755-761.

Quattrini, C., Jeziorska, M., & Malik, R. (2004). Small fiber neuropathy in diabetes: Clinical consequence and assessment. *The international journal of lower extremity wounds, 3*(1), 16-21.

Quattrini, C., et al. (2008). The neuropad test: A visual indicator test for human diabetic neuropathy. *Diabetologia, 51*(6), 1046-1050.

Quattrini, C., et al. (2007). Surrogate markers of small fiber damage in human diabetic neuropathy. *Diabetes, 56*(8), 2148-2154.

Quigley, H. A., Addicks, E. M., & Green, W. R. (1982). Optic nerve damage in human glaucoma: Iii. Quantitative correlation of nerve fiber loss and visual field defect in glaucoma, ischemic neuropathy, papilledema, and toxic neuropathy. *Arch Ophthalmol, 100*(1), 135-146.

Quigley, H. A., Dunkelberger, G., & Green, W. R. (1989). Retinal ganglion cell atrophy correlated with automated perimetry in human eyes with glaucoma. *Am J Ophthalmol, 107*(5), 453-464.

Quigley, H. A., et al. (1995). Retinal ganglion cell death in experimental glaucoma and after axotomy occurs by apoptosis. *Invest Ophthalmol Vis Sci, 36*(5), 774-786.

Realini, T., Lai, M. Q., & Barber, L. (2004). Impact of diabetes on glaucoma screening using frequency-doubling perimetry. *Ophthalmology, 111*(11), 2133-2136.

Reiber, G., Lipsky, B., & Gibbons, G. (1998). The burden of diabetic foot ulcers. *The American journal of surgery, 176*(2), 5S-10S.

Richardson, J. K., & Hurvitz, E. A. (1995). Peripheral neuropathy: A true risk factor for falls. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 50A*(4), M211-M215.

Ritenour, R., Kozousek, V., & Chauhan, B. (2009). The effect of panretinal photocoagulation for diabetic retinopathy on retinal nerve fibre layer thickness and optic disc topography. *Br J Ophthalmol, 93*(6), 838-839.

Rose, G. E. (1988). Clinical assessment of retinal elevations: A review of methods and a novel clinical technique. *Br J Ophthalmol, 72*(9), 703-709.

Roth, J. (1969). Central visual field in diabetes. *Br J Ophthalmol, 53*(1), 16-25.

Roy, M. S., et al. (2004). The prevalence of diabetic retinopathy among adult type 1 diabetic persons in the united states. *Arch Ophthalmol, 122*(4), 546-551.

Ruben, S. T. (1994). Corneal sensation in insulin dependent and non-insulin dependent diabetics with proliferative retinopathy. *Acta Ophthalmol, 72*(5), 576-580.

Sadda, S. R., et al. (2006). Errors in retinal thickness measurements obtained by optical coherence tomography. *Ophthalmology, 113*(2), 285-293.

Said, G. (2007). Diabetic neuropathy-a review. *Nat Clin Pract Neuro, 3*(6), 331-340.

Sampson, G. P., et al. (2012). Visual sensitivity loss in the central 30 degrees of visual field is associated with diabetic peripheral neuropathy. *Diabetologia, 55*(4), 1179-1185.

Sanders, R. D., & Gillig, P. M. (2010). Cranial nerve viii: Hearing and vestibular functions. *Psychiatry (Edgmont), 7*(3), 17-22.

Sato, A., Fukui, E., & Ohta, K. (2010). Retinal thickness of myopic eyes determined by spectralis optical coherence tomography. *Br J Ophthalmol, 94*(12), 1624-1628.

Schatz H, M. D. M. H. J. R. N. (1991). Progressive enlargement of laser scars following grid laser photocoagulation for diffuse diabetic macular edema. *Arch Ophthalmol, 109*(11), 1549-1551.

Scheiner, A. J., Riva, C. E., Kazahaya, K., & Petrig, B. L. (1994). Effect of flicker on macular blood flow assessed by the blue field simulation technique. *Invest Ophthalmol Vis Sci, 35*(9), 3436-3641.

Schuman, J. S. (2008). Spectral domain optical coherence tomography for glaucoma (an aos thesis). *T Am Ophthal Soc, 106*, 426-458.

Scott, T., Foote, J., Peat, B., & Galway, G. (1986). Vascular and neural changes in the rat optic nerve following induction of diabetes with streptozotocin. *Journal of anatomy, 144*, 145.

Sernagor, E., Eglen, S. J., & Wong, R. O. L. (2001). Development of retinal ganglion cell structure and function. *Prog Retin Eye Res, 20*(2), 139-174.

Shabana, N., Pérès, V. C., Carkeet, A., & Chew, P. T. K. (2003). Motion perception in glaucoma patients:: A review. *Surv Ophthalmol, 48*(1), 92-106.

Shahidi, A. M., et al. (2012). Retinal nerve fibre layer thinning associated with diabetic peripheral neuropathy. *Diabet Med, 29*(7), e106-111.

Shimura, M., et al. (2003). Quantifying alterations of macular thickness before and after panretinal photocoagulation in patients with severe diabetic retinopathy and good vision. *Ophthalmology, 110*(12), 2386-2394.

Shoshani, Y. Z., et al. (2011). Contrast sensitivity, ocular blood flow and their potential role in assessing ischaemic retinal disease. *Acta Ophthalmol, 89*(5), e382-e395.

Shun, C. T., et al. (2004). Skin denervation in type 2 diabetes: Correlations with diabetic duration and functional impairments. *Brain : a journal of neurology, 127*(Pt 7), 1593-1605.

Sima, A. A. F., & Kamiya, H. (2006). Diabetic neuropathy differs in type 1 and type 2 diabetes. *Ann Ny Acad Sci, 1084*(1), 235-249.

Sjostrand, J., & Frisen, L. (1977). Contrast sensitivity in macular disease. A preliminary report. *Acta Ophthalmol (Copenh), 55*(3), 507-514.

Smieja, M., et al. (1999). Clinical examination for the detection of protective sensation in the feet of diabetic patients. *J Gen Intern Med, 14*(7), 418-424.

Stavrou, E. P., & Wood, J. M. (2005). Central visual field changes using flicker perimetry in type 2 diabetes mellitus. *Acta Ophthalmol Scand, 83*(5), 574-580.

Stem, M. S., & Gardner, T. W. (2013). Neurodegeneration in the pathogenesis of diabetic retinopathy: Molecular mechanisms and therapeutic implications. *Current medicinal chemistry, 20*(26), 3241-3250.

Steward, J. M., & Cole, B. L. (1989). What do color vision defectives say about everyday tasks? *Optom Vis Sci, 66*(5), 288-295.

Stewart, J. D., Low, P. A., & Fealey, R. D. (1992). Distal small fiber neuropathy: Results of tests of sweating and autonomic cardiovascular reflexes. *Muscle & nerve, 15*(6), 661-665.

Sunness, J. S. (1988). The pregnant woman's eye. *Surv Ophthalmol, 32*(4), 219-238.

Swanson, W. H., Sun, H., Lee, B. B., & Cao, D. (2011). Responses of primate retinal ganglion cells to perimetric stimuli. *Invest Ophthalmol Vis Sci, 52*(2), 764-771.

Szlyk, J. P., Mahler, C. L., Seiple, W., Edward, D. P., & Wilensky, J. T. (2005). Driving performance of glaucoma patients correlates with peripheral visual field loss. *J Glaucoma, 14*(2), 145-150.

Takahashi, A., Nagaoka, T., Sato, E., & Yoshida, A. (2008). Effect of panretinal photocoagulation on choroidal circulation in the foveal region in patients with severe diabetic retinopathy. *Br J Ophthalmol, 92*(10), 1369-1373.

Takahashi, H., et al. (2006). Diabetes-associated retinal nerve fiber damage evaluated with scanning laser polarimetry. *Am J Ophthalmol, 142*(1), 88-94.

Tavakoli, M., et al. (2011). Corneal confocal microscopy detects improvement in corneal nerve morphology with an improvement in risk factors for diabetic neuropathy. *Diabet Med, 28*(10), 1261-1267.

Tavakoli, M., et al. (2010). Corneal confocal microscopy: A novel means to detect nerve fibre damage in idiopathic small fibre neuropathy. *Exp. Neurol., 223*(1), 245-250.

Tekeli, O., Turacli, M. E., Atmaca, L. S., & Elhan, A. H. (2008). Evaluation of the optic nerve head with the heidelberg retina tomograph in diabetes mellitus. *Ophthalmologica, 222*(3), 168-172.

Tesfaye, S., & Boulton, A. (2009). *Diabetic neuropathy*: Oxford University Press, USA

Tesfaye, S., et al. (2005). Vascular risk factors and diabetic neuropathy. *New Engl J Med, 352*(4), 341-350.

Tesfaye, S., et al. (1996). Prevalence of diabetic peripheral neuropathy and its relation to glycaemic control and potential risk factors: The eurodiab iddm complications study. *Diabetologia, 39*(11), 1377-1384.

Thakkar, R. S., et al. (2012). Spectrum of high-resolution mri findings in diabetic neuropathy. *AJR. American journal of roentgenology, 199*(2), 407-412.

Thrall, J. H. (2003). Biomarkers in imaging: Realizing radiologys future. *Radiology, 227*, 633-638.

Tobimatsu, S., Tashima-Kurita, S., Nakayama-Hiromatsu, M., & Kato, M. (1991). Clinical relevance of phase of steady-state veps to p100 latency of transient veps. *Electroencephalography and clinical neurophysiology, 80*(2), 89-93.

Tombran-Tink, J., & Barnstable, C. J. (2007). *Visual transduction and non-visual light perception*: Humana Pr Inc

Tomlinson, D. R. (2002). *Neurobiology of diabetic neuropathy* (Vol. 50): Academic Press

Toprak, I., Yildirim, C., & Yaylali, V. (2012). Optic disc topographic analysis in diabetic patients. *International ophthalmology, 32*(6), 559-564.

Trick, G., Burde, R., Gordon, M., Santiago, J., & Kilo, C. (1988). The relationship between hue discrimination and contrast sensitivity deficits in patients with diabetes mellitus. *Ophthalmology, 95*(5), 693-698.

Ulbig, M., & Hamilton, A. (1993). Factors influencing the natural history of diabetic retinopathy. *Eye (Lond), 7*(2), 242-249.

Ulbig, M., Kampik, A., Thurau, S., Landgraf, R., & Land, W. (1991). Long-term follow-up of diabetic retinopathy for up to 71 months after combined renal and pancreatic transplantation. *Graefes Arch Clin Exp Ophthalmol, 229*(3), 242-245.

Valensi, P., Giroux, C., Seeboth-Ghalayini, B., & Attali, J. R. (1997). Diabetic peripheral neuropathy: Effects of age, duration of diabetes, glycemic control, and vascular factors. *J Diabetes Complications, 11*(1), 27-34.

Valk, G. D., et al. (1997). The assessment of diabetic polyneuropathy in daily clinical practice: Reproducibility and validity of semmes weinstein monofilaments examination and clinical neurological examination. *Muscle & nerve, 20*(1), 116-118.

Van Acker, K., et al. (2009). Prevalence and impact on quality of life of peripheral neuropathy with or without neuropathic pain in type 1 and type 2 diabetic patients attending hospital outpatients clinics. *Diabetes & Metabolism, 35*(3), 206-213.

Van De Poll-Franse, L., Valk, G., Renders, C., Heine, R., & Van Eijk, J. (2002). Longitudinal assessment of the development of diabetic polyneuropathy and associated risk factors. *Diabet Med, 19*(9), 771-776.

van Dijk, H. W., et al. (2009). Selective loss of inner retinal layer thickness in type 1 diabetic patients with minimal diabetic retinopathy. *Invest Ophthalmol Vis Sci, 50*(7), 3404-3409.

van Dijk, H. W., et al. (2010). Decreased retinal ganglion cell layer thickness in patients with type 1 diabetes. *Invest Ophthalmol Vis Sci, 51*(7), 3660-3665.

van Dijk, H. W., et al. (2011). Association of visual function and ganglion cell layer thickness in patients with diabetes mellitus type 1 and no or minimal diabetic retinopathy. *Vision Res, 51*(2), 224-228.

Vaz, J. C. (2011). *Diabetic retinopathy*: World Scientific Publishing Company Incorporated

Ventura, L. M., Golubev, I., Feuer, W. J., & Porciatti, V. (2010). The perg in diabetic glaucoma suspects with no evidence of retinopathy. *J Glaucoma, 19*(4), 243-247.

Verma, A., et al. (2012). Does neuronal damage precede vascular damage in subjects with type 2 diabetes mellitus and having no clinical diabetic retinopathy. *Ophthalmic Res, 47*(4), 202-207.

Verrotti, A., et al. (1998). Relationship between contrast sensitivity and metabolic control in diabetics with and without retinopathy. *Ann Med, 30*(4), 369-374.

Verrotti, A., et al. (2000). Visual evoked potentials in young persons with newly diagnosed diabetes: A long term follow up. *Dev Med Child Neurol, 42*(4), 240-244.

Veves, A., & Malik, R. A. (2007). *Diabetic neuropathy: Clinical management*: Humana Pr Inc

Vibulsreth, S., Hefti, F., Ginsberg, M. D., Dietrich, W. D., & Busto, R. (1987). Astrocytes protect cultured neurons from degeneration induced by anoxia. *Brain research, 422*(2), 303-311.

Vinik, A. (2008). Diabetic neuropathies. In D. LeRoith & A. Vinik (Eds.), *Controversies in treating diabetes* (pp. 109-134): Humana Press.

Vinik, A. I., et al. (1995). Quantitative measurement of cutaneous perception in diabetic neuropathy. *Muscle & nerve, 18*(6), 574-584.

Walker, H. K. (1990). Cranial nerve vii: The facial nerve and taste. In H. W. Walker HK, Hurst JW (Ed.), *Clinical methods: The history, physical, and laboratory examinations* (3 ed., pp. 322). Boston: Butterworths.

Walker, H. K., Hall, W. D., Hurst, J. W., & Walker, H. K. (1990). Cranial nerve v: The trigeminal nerve. In H. W. Walker HK, Hurst JW (Ed.), *Clinical methods: The history, physical, and laboratory examinations* (3 ed.). Boston: Butterworths.

Watanabe, T., & Raff, M. C. (1988). Retinal astrocytes are immigrants from the optic nerve. *Nature, 332*(6167), 834-837.

Werner, J. S., Keltner, J. L., Zawadzki, R. J., & Choi, S. S. (2011). Outer retinal abnormalities associated with inner retinal pathology in nonglaucomatous and glaucomatous optic neuropathies. *Eye (Lond), 25*(3), 279-289.

Wolf-Schnurrbusch, U. E. K., et al. (2009). Macular thickness measurements in healthy eyes using six different optical coherence tomography instruments. *Invest Ophthalmol Vis Sci, 50*(7), 3432-3437.

Wong, A., Chan, C., & Hui, S. (2004). Relationship of gender, body mass index, and axial length with central retinal thickness using optical coherence tomography. *Eye (Lond), 19*(3), 292-297.

Wood, J., Chaparro, A., Carberry, T., & Chu, B. S. (2010). Effect of simulated visual impairment on nighttime driving performance. *Optom Vis Sci, 87*(6), 379-386.

Wood, J. M., Swann, P. G., & Stavrou, E. P. (2000). Visual fields in glaucoma: A clinical overview. *Clin Exp Optom, 83*(3), 128-135.

Woods, R. L., & Wood, J. M. (1995). The role of contrast sensitivity charts and contrast letter charts in clinical practice. *Clin Exp Optom, 78*(2), 43-57.

Wright, K. W., et al. (2007). *Handbook of pediatric neuro-ophthalmology*: Springer

Yagihashi, S., et al. (2001). Neuropathy in diabetic mice overexpressing human aldose reductase and effects of aldose reductase inhibitor. *Brain : a journal of neurology, 124*(Pt 12), 2448-2458.

Yam, J. C. S., & Kwok, A. K. H. (2007). Update on the treatment of diabetic retinopathy. *Hong Kong Medical Journal, 13*(1), 46-60.

Yau, J. W. Y., et al. (2012). Global prevalence and major risk factors of diabetic retinopathy. *Diabetes Care, 35*(3), 556-564.

Yoshiyama, K. K., & Johnson, C. A. (1997). Which method of flicker perimetry is most effective for detection of glaucomatous visual field loss? *Invest Ophthalmol Vis Sci, 38*(11), 2270-2277.

Young, M., Boulton, A., MacLeod, A., Williams, D., & Sonksen, P. (1993). A multicentre study of the prevalence of diabetic peripheral neuropathy in the united kingdom hospital clinic population. *Diabetologia, 36*(2), 150-154.

Young, M. J., Breddy, J. L., Veves, A., & Boulton, A. J. (1994). The prediction of diabetic neuropathic foot ulceration using vibration perception thresholds: A prospective study. *Diabetes Care, 17*(6), 557-560.

Zaslansky, R., & Yarnitsky, D. (1998). Clinical applications of quantitative sensory testing (qst). *J Neurol Sci, 153*(2), 215-238.

Zeimer, R., Asrani, S., Zou, S., Quigley, H., & Jampel, H. (1998). Quantitative detection of glaucomatous damage at the posterior pole by retinal thickness mapping: A pilot study. *Ophthalmology, 105*(2), 224-231.

Zele, A. J., et al. (2008). Adaptation mechanisms, eccentricity profiles, and clinical implementation of red-on-white perimetry. *Optom Vis Sci, 85*(5), 309-317.

Zeng, X., Ng, Y., & Ling, E. (2000). Neuronal and microglial response in the retina of streptozotocin-induced diabetic rats. *Visual neuroscience, 17*(3), 463-471.

Zeppieri, M., Demirel, S., Kent, K., & Johnson, C. A. (2008). Perceived spatial frequency of sinusoidal gratings. *Optom Vis Sci, 85*(5), 318-329.

Zhang, H., & Xu, Y. (2000). The influence on visual evoked potential of panretinal photocoagulation in patients with diabetic retinopathy *Journal of Tianjin Medical University, 4*, 024.

Zhang, X., et al. (2010). Prevalence of diabetic retinopathy in the united states, 2005-2008. *JAMA, 304*(6), 649-656.

Zheng, Y., et al. (2010). Influence of diabetes and diabetic retinopathy on the performance of heidelberg retina tomography ii for diagnosis of glaucoma. *Invest Ophthalmol Vis Sci, 51*(11), 5519-5524.

Zimmet, P. Z., et al. (1994). Antibodies to glutamic acid decarboxylase and diabetes mellitus in the multiple risk factor intervention trial. *Am J Epidemiol, 140*(8), 683-690.

APPENDICES

APPENDIX 1 - ETHICS APPROVAL CERTIFICATE

University Human Research Ethics Committee

HUMAN ETHICS APPROVAL CERTIFICATE

NHMRC Registered Committee Number EC00171

Date of Issue:16/8/11 (supersedes all previously issued certificates)

Dear Prof Nathan Efron

A UHREC should clearly communicate its decisions about a research proposal to the researcher and the final decision to approve or reject a proposal should be communicated to the researcher in writing. This Approval Certificate serves as your written notice that the proposal has met the requirements of the *National Statement on Research involving Human Participation* and has been approved on that basis. You are therefore authorised to commence activities as outlined in your proposal application, subject to any specific and standard conditions detailed in this document.

Within this Approval Certificate are:

* Project Details
* Participant Details
* Conditions of Approval (Specific and Standard)

Researchers should report to the UHREC, via the Research Ethics Coordinator, events that might affect continued ethical acceptability of the project, including, but not limited to:

(a) serious or unexpected adverse effects on participants; and
(b) proposed significant changes in the conduct, the participant profile or the risks of the proposed research.

Further information regarding your ongoing obligations regarding human based research can be found via the Research Ethics website http://www.research.qut.edu.au/ethics/ or by contacting the Research Ethics Coordinator on 07 3138 2091 or ethicscontact@qut.edu.au

If any details within this Approval Certificate are incorrect please advise the Research Ethics Unit within 10 days of receipt of this certificate.

Project Details

Category of Approval:	Administrative Review		
Approved From:	28/05/2008	**Approved Until:**	28/05/2013 (subject to annual reports)
Approval Number:	0800000167		
Project Title:	Ophthalmic markers of diabetic neuropathy		
Experiment Summary:	Employ novel non-invasive ophthalmic markers of peripheral nerve dysfunction to investigate peripheral nerve morphology and function in Type 1 and 2 diabetic patients with and without neuropathy.		

Investigator Details

Chief Investigator:	Prof Nathan Efron

University Human Research Ethics Committee

HUMAN ETHICS APPROVAL CERTIFICATE

NHMRC Registered Committee Number EC00171

Date of Issue:16/8/11 (supersedes all previously issued certificates)

Other Staff/Students:

Investigator Name	Type	Role
Dr Tony Russell	External	Associate Investigator
Ms Nicola Pritchard	Internal	Associate Investigator
Prof John Prins	External	Associate Investigator
Ms Katie P Edwards	Student	Student
Dr Robert Henderson	External	Associate Investigator
Ms Garima Tyagi	Internal	Research Assistant
Ms Ophelia Ho	External	Research Team Member
Ms Kelly Bennett	External	Research Team Member
Mr Andrew Knuckey	Internal	Research Team Member
Ms Jay Lee	Internal	Research Assistant
Dr Andrew Cotterill	External	Associate Investigator
Ms Kath Macintosh	External	Associate Investigator
Ms Sangeetha Srinivasan	Student	Ethics- Student- Course-Doctoral
Ms Colleen Wooten	Internal	Research Team Member
Dr Dimitrios Vagenas	Internal	Associate Investigator
Ms Anne Warne	Internal	Associate Investigator
Dr Geoff Sampson	Internal	Associate Investigator

Participant Details

Participants:
Approximately 220

Location/s of the Work:
Anterior Eye Laboratory, IHBI QUT; Centre for Diabetes and Endocrinology, Princess Alexandra Hospital; Cardiovascular and Endocrine Sciences, Manchester Royal Infirmary

Conditions of Approval

Specific Conditions of Approval:
No special conditions placed on approval by the UHREC. Standard conditions apply.

Standard Conditions of Approval:
The University's standard conditions of approval require the research team to:

1. Conduct the project in accordance with University policy, NHMRC / AVCC guidelines and regulations, and the provisions of any relevant State / Territory or Commonwealth regulations or legislation;

2. Respond to the requests and instructions of the University Human Research Ethics Committee (UHREC);

3. Advise the Research Ethics Coordinator immediately if any complaints are made, or expressions of concern are raised, in relation to the project;

4. Suspend or modify the project if the risks to participants are found to be disproportionate to the benefits, and immediately advise the Research Ethics Coordinator of this action;

5. Stop any involvement of any participant if continuation of the research may be harmful to that person, and immediately advise the Research Ethics Coordinator of this action;

6. Advise the Research Ethics Coordinator of any unforeseen development or events that might affect the continued ethical acceptability of the project;

Date of Issue:16/8/11 (supersedes all previously issued certificates)

7. Report on the progress of the approved project at least annually, or at intervals determined by the Committee;

8. (Where the research is publicly or privately funded) publish the results of the project is such a way to permit scrutiny and contribute to public knowledge; and

9. Ensure that the results of the research are made available to the participants.

Modifying your Ethical Clearance:
Requests for variations must be made via submission of a Request for Variation to Existing Clearance Form (http://www.research.qut.edu.au/ethics/forms/hum/var/var.jsp) to the Research Ethics Coordinator. Minor changes will be assessed on a case by case basis.

It generally takes 7-14 days to process and notify the Chief Investigator of the outcome of a request for a variation.

Major changes, depending upon the nature of your request, may require submission of a new application.

Audits:
All active ethical clearances are subject to random audit by the UHREC, which will include the review of the signed consent forms for participants, whether any modifications / variations to the project have been approved, and the data storage arrangements.

End of Document

APPENDIX 2 – PARTICIPANT INFORMATION AND CONSENT FORM

Princess Alexandra Hospital
Health Service District

Participant Information and Consent Form

for a joint project by Princess Alexandra Hospital

and Queensland University of Technology

Project Title (official): Ophthalmic Markers of Diabetic Neuropathy

Project Title (simplified): Examining the eyes to diagnose nerve problems in patients with diabetes.

Principal Researcher: Prof Nathan Efron[1]

Associates: Prof Andrew Boulton[2], Prof Rayaz Malik[2], Prof John Prins[3], Dr Anthony Russell[3], Nicola Pritchard[1] , Katie Edwards[1] , AProf Andrew Cotterill[4]

1 Queensland University of Technology, 2 University of Manchester, 3 Princess Alexandra Hospital, 4 Mater Children's Hospital

1. Introduction

You (or your child or the person you are responsible for) are invited to take part in this research project. This is because you (or your child or the person you are responsible for) are in the age range of 14-75 years and either have a history of diabetes, or have no history of disease that might affect the nerves of the eye or the body. People who have had eye injury or surgery, other eye diseases (e.g. glaucoma), other general health diseases which may affect the front 'clear window' of the eye, known as the cornea (e.g. keratoconus) or body (e.g. carcinoma, leukemia), large fibre neuropathy (damage to the large nerve fibres), congestive heart failure (weakening of the hearts pumping ability), major mental health problems, HIV-AIDS or diabetic foot ulcer or infection, or those participating in any other research trial will not be eligible.

The research project is aiming to investigate relationship between the nerves of the eye and a condition which involves the peripheral nerves of the body in people with and without diabetes. We hope to determine if some of the measures of the nerves in the eye and the sensitivity of the eye are reduced in people with peripheral nerve damage due to diabetes.

This Participant Information and Consent Form tells you (and your child or the person you are responsible for) about the research project. It explains the procedures involved. Knowing what is involved will help you (or your child or the person you are responsible for) decide if you (or they) want to take part in the research.

Please read this information carefully. Ask questions about anything that you (or your child or the person you are responsible for) don't understand or want to know more about. Before deciding whether or not to take part, you (or they) might want to talk about it with a relative, friend or healthcare worker.

Participation in this research is voluntary. If you (or your child or the person you are responsible for) don't wish to take part, you (or they) don't have to. You (or your child or the person you are responsible for) will receive the best possible care whether you (or they) take part or not.

If you (or your child or the person you are responsible for) decide you (or they) want to take part in the research project, you (or they) will be asked to sign the consent section. By signing it you (or they) are telling us that you (or they):

- understand what you (or they) have read;
- consent to take part in the research project;
- consent to participate in the research processes that are described;
- consent to the use of your (or their) personal and health information as described

You (or your child or the person you are responsible for) will be given a copy of this Participant Information and Consent Form to keep.

If you are the parent or guardian of a child or young person, as the 'person responsible' for the patient, you are invited to consider the patient's participation in this research project. Both the child/young person and the 'person responsible' must consent to participation in the study. If you (or they) decide to take part and later change your mind, you (or they) are free to withdraw from the project at any stage for any reason (stated or unstated) without comment or penalty.

2. What is the purpose of this research project?

This research project focuses on patients with different types of diabetes. As you (or your child or the person you are responsible for) may know, diabetes is associated with high sugar levels in the blood due to the body not producing enough insulin to convert this sugar into energy. We think there might be some differences in the nerves of the eyes of people who have different types of diabetes and we can measure this by using new, simple methods that measure the actual nerves and nerve function. These are the eye tests: corneal confocal microscopy (CCM; high magnification microscope) can be used to look at the nerves in the front of the eye; and corneal non-contact aesthesiometry (NCCA) is used to measure the sensitivity of the front of the eye; ocular coherence tomography (OCT) is used to assess the nerves and tissues at the back of the eye and flicker perimetry (FP) measures how well you can see dim lights (both these techniques are described in Section 3). The measures of nerves and nerve function made by these techniques are thought to be related to diabetic neuropathy, the damage of nerves in the peripheral limbs associated in some patients with diabetes. In the research project we aim to investigate the following:

- Changes in corneal (front of eye) nerve counts and corneal sensitivity over time.
- Changes in retinal (back of eye) nerve layer thickness and sensitivity to light over time.
- The relationship between the progression of nerve damage with the results of other traditional nerve tests such as electrophysiology, measuring electrical signals from the body), measuring how easily you can detect vibration and temperature sensitivity and assessment of level of pain and discomfort in people with different types of diabetes.
- The ability of these eye tests to detect nerve damage earlier than traditional means.
- Identify risk factors associated with changes in nerves and nerve function in people with different types of diabetes; these may include age, height, weight, duration of diabetes, blood pressure, smoking, and poor blood-sugar control.

Understanding these aspects of the nerves may provide healthcare professionals with a quick, simple, cost-effective and repeatable means to identify patients at risk, anticipate and monitor deterioration, and assess new treatments.

Diabetic nerve damage is a significant clinical problem that currently has no effective treatment, and in advanced cases, it is a major cause of ill-health and death worldwide. If left unmanaged, diabetic nerve damage can lead to foot ulceration and ultimately, in some cases, foot amputation. It is therefore important to have the capacity to detect this condition early, monitor its progression and assess the benefits of any treatments.

The results of this study will develop a better understanding of small fibre peripheral nerves in the arms and legs in patients suffering from diabetic nerve damage, and will determine the extent to which these changes are associated with the clinical signs and symptoms of the condition. The significance of this study is that it will reveal the potential for these eye tests to serve as sensitive, rapid, repeatable, 'patient-friendly' eye tests for the detection, diagnosis and monitoring of the progression of diabetic nerve damage. This information will provide a sound basis for the design of trials of treatments for diabetic nerve damage. Data will also be generated which will reveal the importance (or otherwise) of blood sugar control and other metabolic abnormalities and lifestyle factors which may impact on the progression of nerve damage in diabetic patients.

A total of 298 participants will take part in this study at the Institute of Health and Biomedical Innovation (IHBI) at QUT in Brisbane and a further 202 at the University of Manchester in the United Kingdom.

Five groups of people will be recruited in Brisbane:

Group 1: Patients with Type 1 diabetes and without nerve damage

Group 2: Patients with Type 1 diabetes with nerve damage

Group 3: Patients with latent autoimmune diabetes in adults (LADA; similar to Type 1 diabetes but occurring later in life) with nerve damage

Group 4: Patients with Type 2 diabetes with and without nerve damage

Group 5: Non-diabetic participants without nerve damage.

Some of the results of this research will be used by the researchers Ayda Moavenshahididi and Nicola Pritchard to obtain Doctor or Philosophy degrees.

This research is a collaborative project between researchers at QUT, Princess Alexandra Hospital (PAH) and University of Manchester (UM). It has been initiated by the investigators Professors Nathan Efron (QUT), Rayaz Malik, Andrew Boulton (UM), and John Prins (PAH); Dr Anthony Russell (PAH) and optometrists Nicola Pritchard and Dr Katie Edwards (QUT).

This research has been funded in part by the Juvenile Diabetes Research Foundation International and Australia's National Health & Medical Research Council and the George Weaber Foundation (to support Ms Moavenshahidi).

3. What does participation in this research project involve?

Your participation (or that of your child or the person you are responsible for) will involve asking you (or they) to reveal eye and past medical problems, and undergo an examination of the front part of the eye using a high powered microscope, read letters on an eye chart, and have the pressure of the eyes measured. We will ask you (or your child or the person you are responsible for) to complete a questionnaire about pain in your (or their) lower limbs, and undergo simple tests of your (or their) sensations of pain/touch, vibration and temperature. The tests are quick and involve use of a pointed tip, a tuning fork and warm and cool metal rods to test these three sensations. The presence of absence of the reflexes in your knees and ankles using a small hammer will be tested. Your (or their) height, weight and blood pressure will also be measured and a picture will be taken of the back of the eye.

Another high powered microscope, known as a corneal confocal microscope (CCM) will be used to examine the number of nerves at the front part of the eye, the cornea. A drop of anaesthetic is applied to numb the front of the eye and you (or they) will be asked to sit at an instrument and look a target while several images are captured. Initially the drop may sting for 1 or 2 seconds. Because the drop numbs the eye it is possible to scratch the eye without noticing it. Therefore please do not rub the eyes for at least 45 minutes after the drop has been placed in the eye.

Another test of your (or their) ability to feel different sensations will be done using an instrument that can measure when you (or they) just notice sensations of cool, warm and vibration on the foot. For example, for the coolness test you (or they) may feel like "a pulse of cooling" has touched the foot. It is important that before these tests no sedatives, tranquillisers, opiates, or stimulants have been taken in the preceding 12 hours, and not more than one hot drink has been consumed prior to the test.

Another test that can reveal alterations to the nerves is a test of heart rate variability. A measure of heart rate variability will also be conducted to show how the heart responds to deep breathing and to changes in blood pressure and posture.

Corneal non-contact aesthesiometry (NCCA) will be conducted to measure the sensitivity of the cornea. The smallest noticeable air pressure is determined by directing gentle, almost imperceptible puffs of air to the eye, and you (or they) indicate whether the air on the eye can be felt or not. We will also take a small sample of tears (50µl) to examine the proteins; this involves holding a tiny glass tube near the eye for a few seconds. A swab of the conjunctiva (the white part of the eye) will be taken to investigate how diabetes affects the normal microbiota (microscopic living organisms) of the eye.

The speed the nerves conduct messages will also be tested as a measure of nerve damage. Nerve conduction velocity will be measured by putting sensors on the ankle, wrist and elbow. The limb will be kept warm with a heat lamp if necessary. A small electrical current will be applied to the sensor which may feel like a tingling sensation and it may be uncomfortable for you (or them). You (or they) should feel no discomfort once the test is finished.

Ocular coherence tomography (OCT) involves having a drop inserted into one eye to dilate the pupil. Then you (or they) will be asked to fixate a target while seated at the instrument, and at least two OCT images are captured. A photograph of the back of the eye will also be taken using a specialised digital camera. Due to the increased size of the pupil, your (or their) sensitivity to glare may be increased for 4 to 6 hours, so you (or they) may wish to wear dark glasses when outside and/or have someone drive or escort you (or them) home.

Flicker perimetry (FP) involves viewing a light stimulus of varying intensity, and sometimes flickering, which appears in different parts of the visual field. You (or your child or the person you are responsible for) will be required to click a button if you (or they) see the light while looking at a central spot.

At the end of the study procedures the eye will be examined again; follow-up appointments will be made if the investigator believes it is in your (or their) best interests. This study will be carried out at IHBI at QUT.

We expect the visit will be approximately 3 to 5 hours at IBHI at QUT, Kelvin Grove at a time suitable to you. You (or they) will not be paid for participation in this research, but will be provided transport to and from QUT (e.g. parking / vouchers for petrol or cab vouchers will be provided up to approximately $40) and will receive light refreshments during the visit (approximate value $10).

4. What will happen to my test samples?

You (or your child or the person you are responsible for) will be asked to provide consent for the collection of your (or their) blood (approximately 30-35ml, or 3-4 tubes) and urine (approximately 10ml) during the research project. From these samples the levels of protein, glucose, lipid and a test for antibodies for glutamic acid decardoxylase (GADAb) and antibodies to islet cells (ICAb) will be determined and recorded. This will help investigators decide which group to assign you (or them) to. All samples will be individually identifiable at the time of collection, analysis and report; a re-identifiable code will be assigned your (or their) blood results. All blood and urine samples will be assessed through a contracted pathology service and samples are usually destroyed 7 days after collection. Separate consent will be obtained regarding storage of blood samples. Unused tear and swab samples will be destroyed typically within 7 days of collection.

5. What are the possible benefits?

There will be no direct benefit to you (or your child or the person you are responsible for) from your (or their) participation in this research. However, it may benefit the many people who have problems with diabetic neuropathy, because with these instruments and techniques we are able to look at the tissues of the eye under very high magnification. Also these new technologies may reveal features that have not, to date, been discovered but which might serve as sensitive, rapid and useful techniques for the detection, quantification and monitoring of the progression of nerve disease in patients with diabetes as well as other diseases where the nerves of the body are affected. Some people find the opportunity to learn and be a part of something new an interesting experience.

We can provide you (or your child or the person you are responsible for) with state-of-the-art images of your (or their) eye if you (or they) would like them.

6. What are the possible risks?

The risks associated with participation in this study are minimal, and similar to routine diabetic and primary eye care. Minimal scratching the front surface of the eye can occur with corneal confocal microscopy, similar to that which might occur if you (or they) rub the eyes too hard; however, in our experience it is like that noted with normal daily wear of contact wearers. This type of abrasion heals quickly, without intervention, typically within 12 hours.

Having a blood taken may cause some discomfort or bruising. Sometimes, the blood vessel may swell, or blood may clot in the blood vessel, or the spot from which tissue is taken could become inflamed. Rarely, there could be a minor infection or bleeding. If this happens, it can be easily treated.

Nerve conduction tests involve applying a small electrical current to the limb which may feel like a tingling sensation; this may be uncomfortable for you (or them). You (or they) should feel no discomfort once the test is finished.

If you (or your child or the person you are responsible for) become upset or distressed as a result of your (or their) participation in the research, the researcher is able to arrange for counselling or other appropriate support. Any counselling or support will be provided by staff who are not members of the research team. In addition, you (or they) may prefer to suspend or end participation in the research if distress occurs without comment or penalty.

There may be additional risks that the researchers do not expect or do not know about. Tell a member of the research team immediately about any new or unusual symptoms that you (or they) get.

7. What if new information arises during this research project?

During the research project, new information about the risks and benefits of the project may become known to the researchers. If this occurs, you (or your child or the person you are

responsible for) will be told about this new information and the researcher will discuss whether this new information affects you (or them).

8. Can I have other treatments during this research project?

It is important to tell your (or their) doctor and the research staff about any treatments or medications you (or they) may be taking, including over-the-counter medications, vitamins or herbal remedies, acupuncture or other alternative treatments. You should also tell your (or their) doctor and the researchers about any changes to these during participation in the research.

9. Are there alternatives to participation?

Since this study does not involve any treatments, you (or your child or the person you are responsible for) will receive the best possible care whether you (or they) take part or not. Participation in the study does not replace full eye or medical care. You (or they) may also request that your (or their) general practitioner be informed of participation in the study.

10. Do I have to take part in this research project?

Participation in any research project is voluntary. If you (or they) do not wish to take part you (or they) don't have to. If you (or they) decide to take part and later change your mind, you (or they) are free to withdraw from the project at any stage for any reason (stated or unstated) without comment or penalty.

The decision whether to take part or not to take part, or to take part and then withdraw, will not affect your (or their) routine treatment, your relationship with those treating you (or them), nor your (or their) relationship with Princess Alexandra Hospital or Queensland University of Technology.

11. What do I need to do if I decide to withdraw from this research project?

If you (or your child or the person you are responsible for) decide to withdraw, please notify a member of the research team before you (or they) withdraw.

If you (or they) decide to leave the project, the researchers would like to keep the personal and health information about you (or them) and your (or their) blood results that have been collected. This is to help them make sure that the results of the research can be measured properly. If you (or they) do not want them to do this, you (or they) must tell them before joining the research project.

12. Could this research project be stopped unexpectedly?

There are no foreseeable reasons why this research project would be terminated before completion. In the unlikely event this did occur, you (or they) will be informed in writing and asked to attend a final study visit.

13. How will I be informed of the results of this research project?

The research team will provide regular newsletters on the progress of the study. You (or your child or the person you are responsible for) will also receive a copy of any publications that are generated as a result of this study. We expect this research project to be completed in approximately 5 years and a full summary of the results will be provided to you (or them) then. Results from the tests we perform will be sent, with your (or their) permission, directly to your (or their) medical practitioners.

We would like to receive your feedback about your participation and may ask you (or the person you're responsible for) to complete a questionnaire after the visit. These responses will be matched to your visit dates by a team member who has no interaction with you. This

means that the results of these questionnaires will be anonymous to the staff you meet at your visits.

14. What else do I need to know?

Any information obtained in connection with this research project that can identify you (or your child or the person you are responsible for) will remain confidential and will only be used for the purpose of this research project. It will only be disclosed with your (or their) permission, except as required by law. Information about you (or them) may be obtained from your (or their) health records held at PAH (where applicable) for the purposes of this research e.g. additional blood results related to your (or their) PAH clinic visit. If you attend another clinic we will seek your (or the person you're responsible for) permission to obtain your (or their) blood results from your (their) doctor.

Data is stored on paper records in locked filing cabinets at QUT, and the data in electronic form (i.e. entered into a computer) is only available to the research team members and is kept secure by using password-protected limited-access environment to protect your privacy. Data is stored during the project in an identifiable format i.e. with your name attached. In any publication and/or presentation, information will be provided in such a way that you (or they) cannot be identified, except with your (and/or their) permission. This will be done by only using the code number assigned to you (or them) for the purpose of this study; this will provide anonymity.

At completion of the project your (or their) data will be decoded, or de-identified, such that it will not be possible to determine which data belong to which participant. Data for this project will be kept for a minimum of 15 years or 5 years after the last publication, and de-identified data may be shared with national and international data registries. Paper files will,be shredded.

Information about your (or their) participation in this research project may be recorded in your (or their) health records.

How can I access my information?

In accordance with relevant Australian privacy and other relevant laws, you have the right to access the information collected and stored by the researchers about you (or your child or the person you are responsible for). You also have the right to request that any information, with which you disagree, be corrected. Please contact one of the researchers named at the end of this document if you (or they) would like to access your (or their) information.

What happens if I am injured as a result of participating in this research project?

If you (or they) suffer an injury as a result of participating in this research project, hospital care and treatment will be provided by the public health service at no extra cost to you (or them) if you (or they) elect to be treated as a public patient.

Is this research project approved?

The ethical aspects of this research project have been approved by the Human Research Ethics Committee of the Princess Alexandra Hospital and Queensland University of Technology.

This project will be carried out according to the National Statement on Ethical Conduct in Human Research (2007) produced by the National Health and Medical Research Council of Australia. This statement has been developed to protect the interests of people who agree to participate in human research studies.

15. Consent

I have read, or have had read to me in a language that I understand, this document and I understand the purposes, procedures and risks of this research project as described within it.

I give permission for my doctors, other health professionals, hospitals or laboratories outside this hospital to release information to Queensland University of Technology concerning my health and treatment that is needed for this project. I understand that such information will remain confidential.

I consent to the use of blood samples taken from me for use in this specific research project only, as described in Section 4 of this document.

I have had an opportunity to ask questions and I am satisfied with the answers I have received.

I freely agree to participate in this research project as described.

I understand that I will be given a signed copy of this document to keep.

Participant's name (printed) _____

Signature _____ Date _____

Declaration by parent, guardian or person responsible (where appropriate): I agree for my child/young person or the person named above who I am responsible for to participate in this research and I believe that they have understood the explanation of the study, its procedures and risks.

Name of parent/guardian to participant's (printed) _____

Signature _____ Date _____

Name of witness to participant's signature (printed) _____

Signature _____ Date _____

Declaration by researcher*: I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Researcher's name (printed) _____

Signature _____ Date _____

* A senior member of the research team must provide the explanation and provision of information concerning the research project.

Note: All parties signing the consent section must date their own signature.

16. Who can I contact?

Who you (or your child or the person you are responsible for) may need to contact will depend on the nature of your (or their) query; therefore, please note the following:

For further information or appointments:

Landmark Study Email: landmark@qut.edu.au or Katie Edwards, Ph: 07 3138 6154, Email: katie.edwards@qut.edu.au.

If you (or they) have any medical problems which may be related to your (or their) involvement in the project (for example, any side effects), you can contact Dr Anthony Russell Ph: 07 3240 5914 If you (or they) want any further information concerning this project you can contact the following people:

Katie Edwards	Nicola Pritchard	Prof.Nathan Efron
Ph: 07 3138 6154	Ph: 07 3138 6414	Ph: 07 3138 6401
Email: katie.edwards@qut.edu.au	E-mail: n.pritchard@qut.edu.au	E-mail: n.efron@qut.edu.au

If you (or they) feel emergency medical care is required, then go to the nearest hospital Emergency Department.

For complaints:

If you (or they) have any complaints about any aspect of the project, the way it is being conducted or any questions about being a research participant in general, then you (or they) may contact:

Ethics Manager	QUT Research Ethics Officer
Princess Alexandra Hospital Human Research Ethics Committee	Queensland University of Technology Human Research Ethics Committee
Ph: (07) 3240 5856	Ph: (07) 3138 2340
Email: PAH_Ethics_Research@health.qld.gov.au	E-mail: ethicscontact@qut.edu.au

Researcher Ethics Officers/Managers are not connected with the research project and can facilitate a resolution to your (or their) concern in an impartial manner.